SOME CHEMICAL AND PHYSICAL ASPECTS OF EXPERIMENTALLY-INDUCED PULMONARY OEDEMA

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SUMMARY

Section 1.

The effects on pulmonary prostaglandin (PG) synthesis of pulmonary hypertension and oedema has been studied in an isolated, perfused lung preparation and in the intact animal.

In order to detect prostaglandin-like substances (PGLS), 10 ml/min of the venous effluent from the lungs (or carotid arterial blood in intact animals) superfused a series of smooth muscle tissues sensitive to prostaglandins and thromboxane A_2 : rat stomach strip (RSS), rat colon (RC), chick rectum (CR) and rabbit aorta (RbA). Tissues were pretreated with antagonists of catecholamines, histamine, serotonin and acetylcholine, and were calibrated with standard doses of PGE_2 and $PGF_{2\alpha}$.

Nineteen pairs of isolated lungs of rabbit, cat and guinea-pig were perfused with plasma, whole blood, Krebs Ringer solution (KR) or 2.7% dextran T70 in KR (KRD) in a recirculating system. Left atrial pressure (P_{LA}) was increased by between 10 and 30 mmHg for periods of 3-59 min. Gross alveolar oedema developed in all cases. Only in the (3) lungs perfused with KRD was there any release of PGLS. Neither pulmonary hypertension nor subsequent oedema caused release of PGs into the venous effluent in plasma, whole blood or KR perfused lungs.

The lack of PG synthesis in oedema in isolated lungs was confirmed using radioimmunoassay of $PGF_{2\alpha}$ in perfusate, tracheal foam and rapidly-frozen lung tissue.

The effect of dextran T70 (KRD) on pulmonary synthesis of PGs was also studied.

Twelve pairs of isolated rabbit lungs were perfused with KR, KRD or horse plasma in a recirculating system. The lungs were ventilated with 5% CO_2 in air.

In 6 lungs perfused with KRD, PG-like substances corresponding to 0.5-1 ng/ml of PGE_2 appeared in the venous effluent after 60-150 min of perfusion. Lungs perfused with KR or horse plasma did not release PGs during perfusion for up to 4 hours.
When dextran-perfused lungs had increased their basal output of PG, stimuli which normally do not release PGs caused an additional, transient, release of PGs.
Indomethacin added to the perfusate inhibited the observed contractions of the assay tissues, confirming that they were caused by synthesis of PGs.
Mepyramine maleate, a blocker of histamine (H_1)-receptors, but not metiamide, an H_2-receptor blocker, inhibited the PG-release during dextran perfusion. This indicates that PG-release is secondary to liberation of histamine and H_1-receptor activation.

In 9 intact anaesthetized cats increased pulmonary hydrostatic pressure (and occasionally oedema) was induced by inflating a balloon catheter sewn into the left atrium.
P_{LA} was elevated (18 times) to 21-49 mmHg for between 2 and 26 min.
In each animal release of PGLS was detected.
In a further 3 experiments the pulmonary degrading capacity for exogenously-infused PG was shown to be unaltered during and after periods of elevated P_{LA}.
The possible relevance of these findings in pulmonary hypertension is discussed, with special reference to the effects of PGs on the systemic circulation.

Section 2.

Using the bioassay method, experiments were performed to see if release from lungs of PGLS caused the pulmonary vaso- and broncho-

constriction which occurs after experimentally-induced pulmonary microembolism.

Repeated episodes of platelet aggregation were made by i.v. infusions of collagen in anaesthetized cats ventilated at constant tidal volume.

Collagen infusions caused lung responses, as judged from the rise in peak tracheal pressure, and in most cases a transient systemic hypotension also. PGLS were detected simultaneously — but only for as long as lung responses could be elicited.

The role of PGs as mediators of pulmonary smooth muscle constriction after microembolism is discussed.

Section 3.

Pulmonary oedema was quantified and the sequence of oedema formation in isolated rabbit lungs ventilated at constant tidal volume was studied. The relationship between oedema formation and the changes in dynamic lung compliance (C_L) when hydrostatic pulmonary oedema was induced by raising P_{LA} was investigated.

Nineteen lungs were fixed by perfusion with a solution of glutaraldehyde, post-fixed with osmium tetroxide and embedded in epon. They were examined by light microscopy (LM), and alveolar oedema was quantified.

In 22 lungs fixed by rapid freezing, i.e. by immersion in liquid dichlorodifluoro methane (DDM), dried and embedded in paraffin, both interstitial and alveolar oedema were quantified from sections seen in LM by a point-counting method.

A correlation was seen between the amount of alveolar oedema and the fall in C_L observed during perfusion at raised P_{LA} and the relevance of this finding is discussed.

Abbreviations used

PG	prostaglandin
PGLS	prostaglandin-like substances
P_{FA} (or FAP)	femoral arterial pressure
P_{PA}	pulmonary arterial pressure
P_{LA}	left atrial pressure
P_{TP}	tracheal pressure
P_{PI}	peak inspiratory pressure
P_{EE}	end expiratory pressure
CO	cardiac output
RSS	rat stomach strip
RC	rat colon
CR	chick rectum
RbA	rabbit aorta
CJ	cat jejunum
indo	indomethacin
KR	Krebs Ringer
KRD	Krebs Ringer dextran
H_1	histamine$_1$ receptor
H_2	histamine$_2$ receptor
5HT	5-hydroxytryptamine
5HIAA	5-hydroxy indole acetic acid
MAO	monoamine oxidase
NA	noradrenaline
COMT	catechol-O-methyl transferase
Ang I	angiotensin I
Ang II	angiotensin II
ACE	angiotensin converting enzyme
AA	arachidonic acid

RCS	rabbit aorta contracting substance
ECF-A	eosinophil chemotaxic factor of anaphylaxis
NSAID	non steroidal anti inflammatory drug
ACh	acetyl choline
PF_4	platelet factor 4
C_L	dynamic lung compliance
DDM	dichloro-difluoro-methane
DDSA	dodencenyl succinic anhydride

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General Introduction

The lungs are the only organ in the body through which the total cardiac output must be pumped, as they lie in series between the right and left ventricles of the heart. This, and their particular anatomy of extensive branching of the vascular tree and their huge capillary bed, accounts for their suitability as the organ of gas exchange, and also for their more recently discovered important metabolic function (Bakhle & Vane, 1974).

The lung as an organ of gas exchange

A brief outline of the structure and function of the lungs will be given here. Lung anatomy has been extensively studied (von Hayek, 1960; Parker, Horsfield & Cumming, 1971; Staub & Storey, 1962) for example by scanning electron microscopy (Nowell & Tyler, 1971) by serial sectioning for light and electron microscopy (Weibel, 1963) and by three dimensional reconstruction (Ogawa, 1920; Miller, 1937; Angus & Thurlbeck, 1972).

The tissue framework of the lungs is well organized for the uptake of oxygen into and the removal of CO_2 from the blood. In the mature lung airways and blood vessels have a characteristic positional relationship to each other: from the hilum to the septa (periphery) the pulmonary arteries and the airways lie in close proximity while the pulmonary veins lie between two airway trees. For convenience, the lungs can be pictured as three functional zones (Weibel & Gomez, 1962a):

1) The conductive zone consists of bronchi, bronchioles,

pulmonary arteries and veins, where airways and blood vessels have well-developed walls containing smooth muscle, which may play a regulatory role.

2) The transitory zone, where respiratory bronchioles, alveolar ducts and sacs lie in close proximity with the pre- and postcapillaries and where a little gas exchange can take place and

3) The respiratory zone, which contains the alveoli and the alveolar capillaries in such close contact that gas exchange between air and blood takes place.

1. The conductive zone. Air enters the trachea at high velocity but this decreases due to branching of the bronchial tree which increases total cross sectional area. In the terminal lung units fresh inspired gas mixes with gas present in the units only by the kinetic energy of the molecules, and blood gas exchange takes place by diffusion.

2. The transitory zone. Airways (and pulmonary arteries) branch at an acute angle (von Hayek, 1953). The branching structure of the bronchial tree is best described using an order numbering system as used for river branching. In this context, a branch is an airway having a single axial direction. Since it is very asymmetrical, the smallest structures having no alveoli (terminal bronchioles) have order number 1. Where structures of like number join, the "confluence" takes the next higher number and where structures of unlike number join the "confluence" takes the higher of the two numbers and does not increase ("Strahler system").

The order number is directly related to the log of the number of branches. That is, the number of structures increases exponentially with decreasing order number. The same relationship exists between the diameter of branches and order number, and between length and order number.

3. The respiratory zone i.e. the terminal ventilatory unit

Definitions and terminology. A terminal ventilatory unit is defined as that structure supplied by a single terminal bronchiole. Beginning proximally the terminal bronchiolar unit consists of a respiratory bronchiole, alveolar duct and alveolar sac. The terminal bronchiole is that branch in the airways immediately preceeding a branch which contains an alveolus in its wall. Walls of the terminal bronchiole are covered with bronchial epithelium. The first branch containing an alveolus is a respiratory bronchiole(RB), an airway with RB or ducts as major outlets. Its walls have a significant ($> 5\%$) amount of bronchial epithelium and a few alveoli and/or sacs. This is the first gas exchanging structure.

Branching distal to the terminal bronchiole is probably dichotamous: each branch gives rise to two daughter branches. Successive orders of respiratory bronchiole often have increasing numbers of alveoli. An alveolar duct is an air space with several outlets: one or more sacs or ducts and one or more alveoli with limited ($< 5\%$) patches of bronchial epithelium. The alvoli are grouped round a central duct in a helical fashion and there may be several orders of alveolar ducts. An alveolar sac is an airspace with only alveoli as outlets and an alveolus is a terminal airspace

without outlets other than pores of Kohn.

There is an arithmetric progression in the number of structures from the terminal bronchiole to the alveolar sac.

The acinus includes the terminal bronchiole and all other branches of airways (respiratory bronchioles) and air spaces (alveolar ducts, alveolar sacs, and alveoli) distal to the terminal bronchiole. It is defined as that portion oflung parenchyma connected to the first order respiratory bronchiole (Gamsu, Thurlbeck, Macklem & Fraser, 1971) and is the site of gas exchange (Miller, 1937; von Hayek, 1960).

Morphometry of the alveolus

The alveoli exist in a honeycombe-like structure, the walls of which contain the capillary network that is shared between neighbouring alveoli. Alveoli are suspended by connective tissue fibres (Miller, 1937; Haugaard, 1968) that extend between two anchor points: a peripheral (septal) fibre system connected to the pleura and an axial fibre sleeve, in the "wall" of the alveolar duct, continuous with the fibrous wall of the conducting airways. The size of the alveoli is quite homogeneous (Storey & Staub, 1962; Staub, 1963; Klingele & Staub, 1970) although there is a vertical gradient of alveolar size in lungs (Section 3). The shape depends on the degree of inflation. Alveolar size is about 250 μ in human adults, and the lung contains approximately 3×10^8 alveoli (Weibel & Gomez, 1962a & b; Weibel, 1963).

The capillary network forms a continuum throughout large parts

of the lung, extending across several acini and being supplied and drained at short intervals by pre- and post- capillaries (Weibel, 1963).

The structure of the interalveolar septum, which is the air-tissue barrier, has been elucidated by electron microscopy and is composed of three layers: a capillary endothelium, an alveolar epithelium and a very narrow interstitial space (Bensch, 1967; Weibel, 1969; Bartlett & Remmers, 1971).

Capillary endothelium is a single cell layer sheet of squamous cells as in muscle capillaries, with a thin layer of cytoplasm round the nucleus. The cytoplasm contains few organelles but many small pinocytotic vesicles for macromolecular transport (Bensch, Dominguez & Liebow, 1967; Bruns & Palade, 1968; Palade & Bruns, 1968; Schneeberger & Karnovsky, 1968) and may be extremely thin (20 nm) in parts (Weibel, 1972). These cells are metabolically active (see page 7).

Interstitium:

Nearly half the contact surface between capillary endothelium and alveolar epithelium is fused basement membranes of the two cell layers. In parts where basement membranes are separated the space contains collagen elastic fibres (Ross & Benditt, 1965; Low, 1971) and fibroblasts may also be present.

It is possible that mast cells, which are known to exist in the lungs (Riley, 1961; Hill, 1965) lie in the pulmonary interstitium (Conradi, Burri, Kapanci, Robinson & Weibel, 1971; Said, et al., 1968). Leucocytes are only present in pathological conditions. The interstitium of the interalveolar septum is thus very thin and forms a connective fibrous network from the alveolar duct wall to the walls

of small vessels and bronchioles (Weibel, 1967). Sobin, Fung, Tremer & Rosenquist (1972) have postulated a sheet flow model for fluid movement in pulmonary interstitium and have suggested that collagen fibres may lie at right angles to the alveolar septum to form a post supporting, and accounting for the compliance of, the capillary sheet. However, there is no evidence for this in alveolar walls.

The interstitium provides the pathway for fluid drainage from alveolar capillaries to the lymphatics (Fishman & Hecht, 1969) which lie in the connective tissue surrounding small vessels and bronchioles (Staub, 1967; Staub, Nagano & Pearce, 1967; Lauweryns, 1970).

The alveolar epithelium

The alveolar epithelium is made up of three cell types:

1) Type I cells are squamous epithelial cells and provide the lining of the alveolar surface.

2) Type III cells, called alveolar brush cells because of their brush border of microvilli, are not very common. They are cuboidal and do not contain osmophilic granules. A function as a receptor site has been postulated (Meyrick & Reid, 1968).

3) Type II epithelial cells contain osmophilic granules. The alveolar surface of the cell has abundant microvilli and the cell contains many organelles, such as mitochondria, endoplasmic reticulum, ribosomes, and Golgi apparatus. It probably secretes the substances which form the extracellular lining of the alveoli, a thin surface film of mucopolysaccharide and surfactant, a surface-tension reducing monomolecular film of phospholipid (Gil & Weibel, 1969-70). It is

an important lung metabolic cell, as evidenced by its high content of oxidative enzymes (Tyler & Pearse, 1965).

The lung as a metabolic organ

The pulmonary capillary bed has a huge surface area (50 m^2 in adult human) and normally contains 75-100 ml blood, which has the effect of spreading the blood such that red corpuscles pass through slowly in single file. Thus blood is in very close contact with the metabolically-active endothelial cells. Recently attention has been focused on the role of the lung as a metabolic organ (Said, 1968; 1973; Bakhle & Vane, 1974; Junod, 1974; Fishman & Pietra, 1974a 1974 b). The importance of the effects of circulating vasoactive substances must be noted in the context of the lung itself, as well as their effects in the systemic circulation. For example, oxygen tension in arterial blood may be altered by substances which affect the tone of the pulmonary arterioles.

The metabolic function of the lungs consists of three main areas; inactivation, potentiation and secretion.

(i) Inactivation, binding and degredation

Histamine does not appear to be removed from the pulmonary circulation of isolated dog lungs (Eiseman, Bryant & Waltuch, 1964). However, preparations of chopped lungs of guinea-pig and rat are capable of metabolizing histamine (Bennet, 1965). Thus it is important to use perfused lungs to determine the fate of circulating vasoactive substances (Vane, 1969). Johnson (1970) has shown *in vivo* that injected H^3 histamine rapidly disappears from plasma

due to uptake and metabolism and accumulates in various tissues in the body: kidney, liver, ileum, skin and, to a lesser extent, lung, where uptake was into non-mast cell pools. Johnson also showed that tissue uptake and/or inactivation of histamine decreases during anaphylaxis.

5-hydroxytryptamine (5HT) is removed from the pulmonary circulation (Gaddum, 1953) and its metabolite, 5-hydroxy indole acetic acid (5-HIAA) appears in the effluent. This has been shown to occur in many species: rat, cat, dog, rabbit and man (Alabaster & Bakhle, 1970; Gaddum, Hebb, Silver & Swan, 1953; Thomas & Vane, 1967; Gillis & Iwasawa, 1972 and Gillis, Green, Cronau & Hammond, 1972). It is taken up by the lung (Alabaster & Bakhle, 1970) by a carrier mediated transport system into endothelial cells of large vessels and capillaries (Strum & Junod, 1972) and oxidised by monoamine oxidase (MAO).

Noradrenaline (NA) is also removed from the blood by a carrier mediated process and metabolised by MAO and COMT. 96% of infused NA (150 µg to 1 mg) was degraded by the isolated dog lung (Eiseman, Bryant & Waltuch (1964), while blood alone degraded 51%. Uptake is probably extraneuronal (i.e., uptake 2 (Junod, 1974)). However, adrenaline passes through the pulmonary circulation without change. The nucleotides ATP and AMP are dephosphorylated during passage through the lungs, probably by enzymes in caveolae of the endothelial cells of the lumen (Smith & Ryan, 1970; Ryan & Smith, 1971).

(ii) Potentiation

During passage through the lungs the relatively inactive peptide

angiotensin I (Ang I) is converted to angiotensin II (Ang II) (Ng & Vane, 1967, 1968; Fanburg & Glazier, 1973; Ryan, Stewart, Leary & Ledingham, 1970) which is a potent systemic vaso-constrictor. The enzyme complex, angiotensin converting enzyme (ACE), or Kinase II, has been located (Smith & Ryan, 1972; Ryan, Smith & Neimeyer, 1972; Caldwell, Seegal, Hsu, Das & Soffer, 1976) in caveolae, on the surface of the endothelial cells, which open directly into the lumen.

Conversion of Ang I has been reported not to occur in other vascular beds (Ng & Vane, 1968). However, caveoli probably exist on all endothelial surfaces but conversion might occur more rapidly in the lungs due to the slower linear velocity of blood in the lungs allowing longer blood-endothelial cell contact. Angiotensin II is not retained or inactivated in the pulmonary circulation but is eliminated by the systemic circulation (Ng & Vane, 1968).

(iii) Secretion

Lungs are rich in histamine, which is present in mast cells, mainly around small blood vessels and in alveolar walls (Said et al., 1968) and in non-mast cell tissue pools (Johnson, 1970). Histamine is discharged from mast cells of human and rat lung by an IgE-dependent mechanism (Paterson, Leid, Said, Wasserman & Austen, 1976) or from bronchial lumen of human, monkey and dog (Paterson, Ts'ao & Suszko, 1976), and is one of the mediators of the cardiovascular and respiratory responses to antigen - antibody reactions (Bartosch, Feldberg & Nagel, 1932; Dragstedt & Gebauer-Fuelnegg, 1932), to microembolism (Nadel, Colebatch & Olsen, 1964) and probably to hypoxia (Hauge, 1968).

Lungs are also capable of releasing prostaglandins (p. 18) which they synthesize from essential fatty acids (Anggard & Samuelsson, 1965; Bergström, Carlson & Weeks, 1968). Prostaglandins and their metabolites are released during anaphylaxis (Piper & Vane, 1969; Mathé & Levine, 1973; Liebig, Bernauer & Peskar, 1974). Also released during anaphylaxis are 5HT (Rowley & Benditt, 1956; Paratt & West, 1957), adrenaline (Piper, Collier & Vane, 1967), kinins (Brocklehurst & Lahiri, 1962, 1963), slow reacting substance of anaphylaxis (SRS-A; Brocklehurst, 1960) and eosinophil chemotaxic factor of anaphylaxis (ECF-A; Kay, Stechschulte & Austen, 1971).

Section 1. The release of prostaglandins from lungs during increased hydrostatic pressure and oedema

Introduction

Prostaglandin synthesis

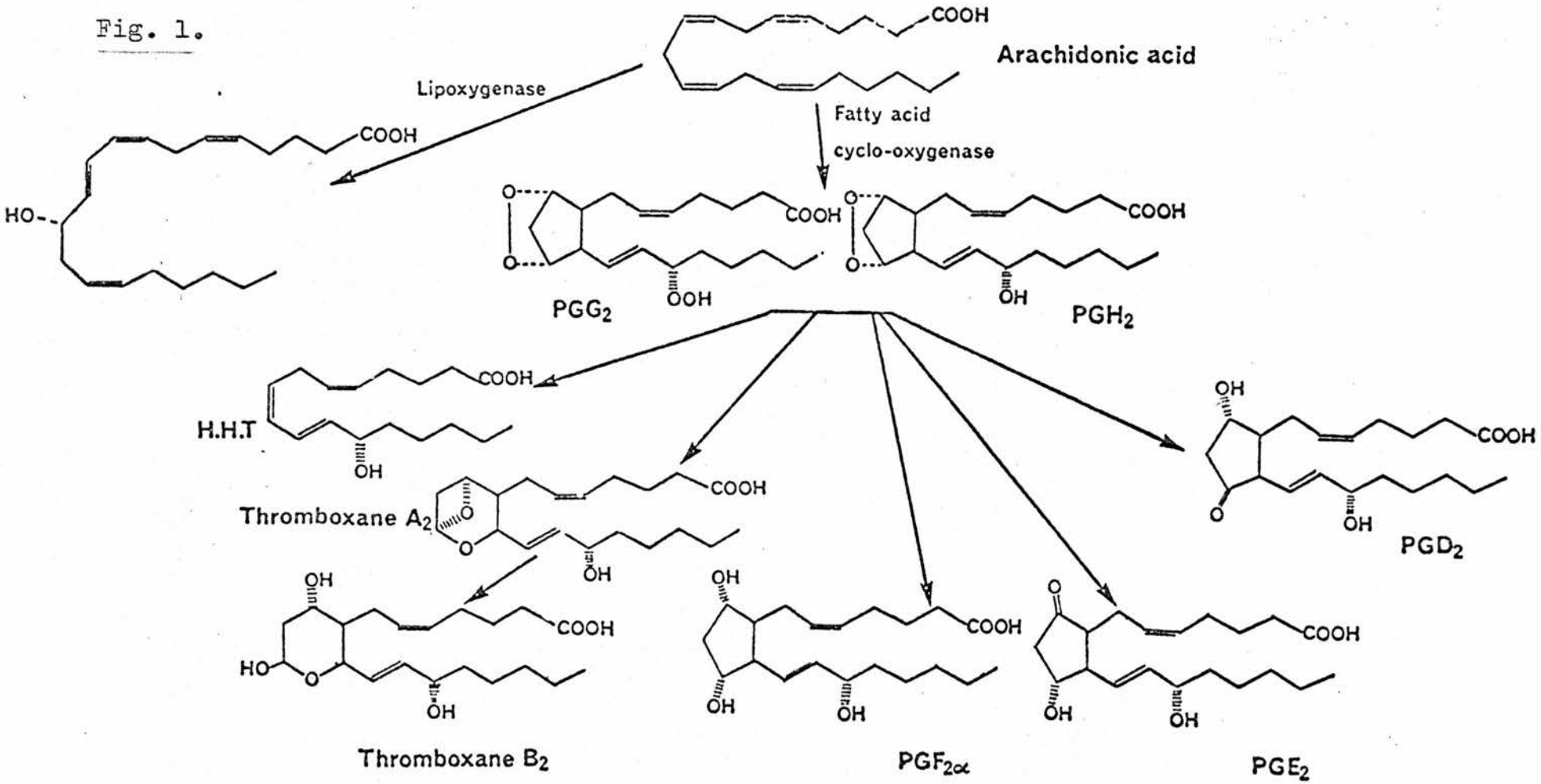
Prostaglandins are 20 carbon fatty acids which contain a cyclopentane ring, and are grouped according to their various substituents at C_9, C_{11} and C_{15} into PGs of the E, F, D, G and H series. PGA, B and C are formed by dehydration of PGE. Each series is further divided into up to 3 groups denoted by the subscripts 1, 2 or 3 according to their degree of saturation of the side chains, i.e. the members of a series with subscript 1 have a double bond at the C_{13} position ($\triangle^{13}$) while subscript 2 means an unsaturated bond at C_5 and C_{13}; and subscript 3 also at C_{17}. (Oesterling, 1974).

Prostaglandins are physiologically active compounds which are present in many animal tissues. Their structures are known and they are formed by enzymatic convertion of the essential fatty acids in many tissues (Ängg̊ard & Samuelsson, 1965; Bergström, Danielsson & Samuelsson, 1964; Dorp, Beerthuis, Nugteren & Vonkeman, 1964).

The pathway for PG synthesis is catalyzed by the enzyme complex, prostaglandin synthetase, associated with the microsomal fraction in almost all mammalian cell types so far tested. The convertion pathways from the essential fatty acid, arachidonic acid are shown in fig.1 while the precursor for the less saturated prostaglandins of subscript 1 is dihomo - γ -linolenic acid. The pathway to PGI_2 from the endoperoxides is not shown.

The pathway of conversion from arachidonic acid to HETE and HHT (both biologically inactive) and to prostaglandins and thromboxane.

Fig. 1.



Endogenous AA is derived from phospholipid by the activity of the enzyme phospholipase A. Arachidonic acid is then converted by the enzyme cyclo-oxygenase to the endoperoxides PGG_2 and via peroxidase to PGH_2 (Hamberg & Samuelsson 1973; Hamberg, Svensson & Samuelsson 1974; Nugteren & Hazelhof, 1973). These cyclic endoperoxides are very unstable, with a half life of about 4 minutes.

The endoperoxides are then converted to PGE_2, $PGF_{2\alpha}$ and PGD_2 by isomerase and peroxidase reactions of the microsomal enzyme system. The type of prostaglandin that predominates in the synthesis depends on the tissue involved. It is interesting to note that the proportion of, for example, PGE to PGF synthesized may be chemically altered (Lands, Lee & Smith, 1971; Nugteren, Beerthuis & van Dorp, 1967).

PGD_2, previously thought to be inactive, is formed during platelet aggregation (Oelz, Oelz, Knapp, Sweetman & Oates, 1977). It is an extremely potent inhibitor of platelet aggregation (Smith, Silver, Ingerman & Kocsis, 1974). PGD_2 and PGD_1 possess opposing actions on vascular permeability in the rat skin (Flower & Kingston, 1975) and PGD_2 has pulmonary and cardiovascular effects (Wasserman, du Charme, Griffin, De Graaf and Robinson, 1977).

The conversion of arachidonic acid may follow another pathway from the endoperoxides, namely conversion by the microsomal enzyme, thromboxane synthetase, to thromboxanes (Hamberg, Svensson & Samuelsson, 1975; Needleman, Moncada, Bunting, Vane, Hamberg & Samuelsson, 1976) (see fig.1). Thromboxane A_2 is highly unstable with a half life of approximately 30 sec. in aqueous medium at 37°C (Samuelsson 1976),

although longer (about 3 min) in plasma (Granstrom, Kindahl & Samuelsson, 1976).

Thromboxane A_2 is so called because it is a very potent platelet aggregating agent (Hamberg, Svensson & Samuelsson, 1975), and is now considered (Samuelsson, 1976) to be the main constituent of the rabbit aorta contracting substance (RCS) released from anaphylactic guinea-pig lungs (Piper & Vane, 1969).

Recently an enzyme was found which transforms the endoperoxides to a new prostaglandin substance, PGI. (Moncada, Gryglewski, Bunting & Vane, 1976). This compound is formed in certain cell layers of the arterial wall, notably in highest concentrations in the vascular endothelium, (Herman, Moncada & Vane, 1977), where it has been postulated to play a role as an endogenous inhibitor of platelet aggregation and preventer of thrombus formation (Moncada, et al., 1976; Moncada, Herman, Higgs & Vane, 1977).

Two other products, HETE and HHT(see fig.1), which appear to possess no biological activity, are formed during the metabolism of arachidonic acid in human platelets (Hamberg, Svensson & Samuelsson, 1974).

Prostaglandins are present in most tissues in small amounts (Karim, Hillier & Devlin, 1968; Karim, Sandler & Williams 1967). When PGs are released the quantities are usually higher and synthesis must be stimulated.

Inhibition of prostaglandin synthetase

Non-steroid anti-inflammatory drugs (NSAID) inhibit the PG synthetase activity of many tissues (Vane 1971; Flower, 1974; Ferreira & Vane, 1974). Indomethacin inhibits the activity of the oxygenase component but not the isomerase component of the prostaglandin synthetase system (Miyamoto, Yamamoto & Hayashi, 1974). Flower & Vane (1972) report that indomethacin in a variety of tissues inhibits synthetase activity to a different extent and this is confirmed by Flower, Cheung & Cushman (1973). Pong & Levine (1976) have shown that the dependent factor is the concentration of the substrate arachidonic acid. The mechanism of action of these drugs has not been completely elucidated, although inhibition must take place at an early stage in synthesis since the production of endoperoxides is inhibited (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974; Hamberg, Svensson & Samuelsson, 1974). Lands, le Tellier, Rome & Vanderhoek (1973) have suggested a mechanism by which the inhibitor could bind to a site on the synthetase complex near to the active site and thus cause inhibition of synthesis.

Metabolism of prostaglandins

Removal from the circulation. In a single passage through the lungs of cat, dog and rabbit about 90% of PGE_1 and PGE_2 is removed from the blood (Ferreira & Vane, 1967a). Also prostaglandin E_1, E_2 and $F_{2\alpha}$ are almost completely inactivated during passage through the pulmonary circulation of cat, dog, rat, guinea-pig and man (Biron, 1968a, McGiff, Terragno, Strand, Lee, Ng & Lonigro, 1969; Horton & Jones, 1969; Piper & Vane, 1969 and Biron 1968b). Prostaglandins of the A_1

series have also recently been shown to be metabolized to a certain extent (Gross & Gillis, 1976), and PGA_2 is 23-75% removed (Piper, Vane & Wyllie, 1970), although neither PGA_1 nor PGA_2 is inactivated in dog lung (McGiff et al, 1969) when estimated by superfusion.

Breakdown

Although prostaglandins are stable in blood (Ferreira & Vane, 1967a) their half life in the circulation is short. They are inactivated by an enzyme complex present intracellularly in the particle-free fraction of cells (Anggard & Samuelsson, 1965), and converted to compounds which are usually less biologically active than the parent ones. They showed that homogenates of guinea-pig lung tissue converted PGE_1 to 15- keto, 13,14- dihydro PGE_1 and 13,14- dihydro PGE_1. PGE_2 and PGE_3 are similarly transformed by oxidation of the secondary alcohol group at C_{15} and/or by reduction of the $\triangle^{13}$- trans double bond (Anggard, Green & Samuelsson 1965, Anggard & Samuelsson 1965.)

In vivo metabolism has been studied in several species. In man, four enzymic processes act to metabolize PGE_2. Initially there is oxidation of the hydroxy group at C_{15} by 15-hydroxyprostaglandin dehydrogenase (15-OH PGDH), then reduction of the trans double bond by prostaglandin $\triangle^{13}$ reductase. This reaction is assisted by the electron withdrawal effect of the keto group previously formed at C_{15}. It is followed by two steps of β - oxidation of the carboxyl side chain and an ω - oxidation reaction. Two additional metabolites have been found during breakdown of $PGF_{2\alpha}$ PGE_1 and $PGF_{1\alpha}$ are converted to the same main metabolites as outlined for PGE_2 and $PGF_{2\alpha}$

(Samuelsson, Granstrom, Green & Hamberg, 1971).

The enzyme capacity of the 2 lungs is considerable: it is only exceeded at an infusion rate of 10 µg/min of PGE_2 (Piper, Vane & Wyllie, 1970). Since they are so rapidly metabolized in lungs, prostaglandins are likely to act either intracellularly, or close to their site of synthesis, i.e. as local, not circulating, hormones (Vane, 1969; Silver & Smith, 1975). However modification of the action of the metabolizing enzymes may occur either chemically or as a function of different physiological and pathological conditions. For instance, prostaglandin dehydrogenase activity has been shown to vary with age in rat lung (Pace-Asciak & Miller, 1973); rabbit lung (Sun & Armour, 1974) rat kidney (Pace-Asciak, 1975) and sheep brain, lung and kidney (Pace-Asciak, 1976). PGDH activity increased to peak at 19 days after birth, then decreased to adult levels in rat kidney and rat lung (Pace-Asciak, 1975; Pace-Asciak & Miller, 1973); while in the maternal rabbit lung enzyme activity near term was approximately 20 times that of the foetus, newborn or adult animal (Sun & Armour, 1974). While confirming this observation, Bedwani & Marley (1974, 1975) have also shown a corresponding increase of the pulmonary inactivation of exogenous PGE_2 in the rabbit during pregnancy.

Decreased PGE metabolism has been found in homogenates of rat lung and kidney during endotoxin shock (Nakano & Prankan, 1973) and in guinea-pig lung homogenates after exposure of the animals to pure oxygen for several hours (Parkes & Eling, 1975).

Cellular uptake of PG is necessary for subsequent metabolism (Ryan, Niemeyer & Ryan, 1975) and this process may be inhibited, for example by the use of chemicals, or drugs, such as diphloretin and

polyphloretin phosphate. (Eling, Hawkins & Anderson, 1977; Crutchley & Piper, 1974, 1975a). Other authors have postulated that PGs may be metabolized in the caveolar sites of the endothelium of the vascular lumen (Jose, Niederhauser, Piper, Robinson & Smith, 1976), but an intracellular site would appear to be favoured by the majority of investigators. The requirement of a carrier mediated mechanism for transport of PGs across the normally impermeable cell membrane has also been postulated (Bito, 1972).

Prostaglandin synthesis and release due to physiological, pathological or chemical stimuli

As outlined by Ramwell & Shaw (1970) and Piper & Vane (1971) virtually all tissues are able to release prostaglandins as a result of various physiological, mechanical and pathological stimuli.

Lungs are also capable of releasing other vasoactive substances (see p. 9) whose release can be affected by prostaglandins and vice versa. For example PGE_2 potentiates the effect of histamine in perfused rabbit kidney and guinea-pig lung (Ercan & Turker, 1975); it also causes release of histamine from skin (Söndergaard & Greaves, 1971). Low doses of PGE_1 have been shown to enhance the release of histamine while depleting cyclic AMP (Tauber, Kaliner, Stechschulte & Austen, 1973) and high doses of PGE_1 inhibit release (Tauber et al., 1973; Kaliner & Austen, 1974). Inhibition of prostaglandin synthetase also inhibits the release of histamine by antigen from rat peritoneal mast cells (Thomas & Whittle, 1976).

Release of prostaglandins from sympathetic nerve stimulation

There is a large body of evidence in favour of the hypothesis that prostaglandins could play a role in the regulation of noradrenaline release to nerve stimulation in many organs, including the spleen (Davies, Horton & Withrington, 1968; Hedqvist, 1970a), heart (Wennmalm, 1976) and pancreas (Hamamdžic & Malik, 1977). The mechanism involved is most likely reduction of transmitter release (Hedqvist 1970b) but a post synaptic action may sometimes be involved (Clegg, 1966). Prostaglandins of the E series are the major type released by nerve stimulation (Hedqvist, 1970b) and they decrease the responses produced by nerve stimulation. However, PGs of the F series facilitate transmission and enhance the effects of noradrenaline (Kadowitz, George, Joiner & Hyman, 1973) in lungs.

Recently Mathe, Tong, Tisher & Yen (1977) have reported release of prostaglandin E from lungs due to sympathetic nerve stimulation.

Release of prostaglandins during platelet aggregation

PGE_2 and $PGF_{2\alpha}$ are released in vitro during aggregation in platelet-rich plasma (Smith & Willis, 1970; Smith, Ingerman, Kocsis and Silver, 1973). In Section 2 (p.28) this release is discussed more fully.

Release from lungs of prostaglandins due to chemical stimuli

Alabaster & Bakhle (1976) have shown release of prostaglandins from isolated perfused rat, guinea-pig and dog lung after infusion of tryptamine, 5-HT, acetyl choline (ACh) and histamine. Release due to 5-HT and tryptamine was antagonized by methisergide, while that due to histamine was blocked by mepyramine, an H_1 receptor blocker.

Release was accompanied by a rise in perfusion pressure which may have been the stimulus for PG release (p. 21). Bradykinin, arachidonic acid and dihomo - γ - linolenic acid also stimulate PG synthesis and release (Palmer, Piper & Vane, 1973). Mathé, Strandberg & Yen (1977), have reported prostaglandin and PG metabolites are released by slow reacting substance in guinea-pig and human lung tissue. Noradrenaline infusion has been reported to release prostaglandins from the vasculature of isolated perfused rabbit ear (Gryglewski & Korbut 1975), but it inhibits anaphylaxis-induced prostaglandin release from guinea-pig lung (Mathe & Levine, 1973).

Release of prostaglandins due to physiological or pathological stimuli

Various physiological and pathological stimuli release PGs, possibly through their common action of cellular distortion (Piper & Vane, 1971). Massage of isolated perfused lungs releases prostaglandins into the perfusate (Piper & Vane, 1971). Tissue distension for example of the rat stomach (Bennet, Friedman & Vane 1967) dog bladder (Gilmore 1968) and human (Horton, Jones, Thompson & Poyser, 1971) or guinea-pig uterus (Poyser, Horton, Thompson & Los, 1971), causes release of prostaglandins . Also Karim (1968) reported release of PG into blood during labour.

The mechanical stretching of lung tissue during hyperventilation releases prostaglandins from guinea-pig lung (Berry, Edmonds & Wyllie, 1971) or from dog lungs (Said, Kitamura & Vreim, 1972). Also chopped lung tissue can be stimulated to release PGs (Palmer, Piper & Vane 1973; Piper & Walker 1973). Release of prostaglandins from lungs has also been reported after embolism by infusion of particles

(Palmer , Piper & Vane 1967; Lindsey & Wyllie, 1970) or by infusion of air (Piper & Vane, 1971). Particle infusion into dog spleen (Gilmore, Vane & Wyllie, 1969) also releases PGs and Piper & Vane (1971) have suggested that the mechanism by which release occurs may be distortion of cells during expansion of the extravascular space, when fluid filtration is induced.

Pulmonary oedema in the early stages involves considerable distension of the extravascular (interstitial) space of the lung by protein and water before fluid overflows into the alveoli (see Section 3). Said & Yoshida (1974) and Chijimatsu, Hara & Said (1976) have reported that prostaglandins, most probably of the F type, are released in considerable amounts (up to 20ng/ml PG $F_{2\alpha}$) into the venous effluent and tracheal fluid of isolated lungs during oedema. Oedema was induced either by prolongation of perfusion or by elevation of outflow pressure, and this would seem to suggest that both stretching of blood vessels during raised outflow pressure and distension of the extravascular space may release PGs.

Release of PGs E and F from splenic capsular strips has been shown to occur after incubation with Ang II (Diekmann, Jobke, Peskar & Hertting, 1977). Since release also occurs after contractions produced by NA or methoxamine (Jobke, Peskar & Hertting, 1976), it is possible that release is due to mechanical stimulation during contraction. However, there may be a modulator role for PGs in the systemic vasoconstriction due to Ang II, since indomethacin potentiates the Ang II - induced contractions (Diekmannet al, 1977) and the vasoconstrictor actions of Ang II in man (Negus, Tannen & Dunn, 1976). Also in rabbit AA has been shown to increase and indomethacin to decrease

plasma renin activity (Larsson, Weber and Änggård, 1974).

However, not all stimuli which cause mechanical distension lead to release of PGs. ATP causes a decrease in perfusion pressure (Minkes, Douglas & Needleman, 1973), as does the vasodilator bradykinin (Messina, Weiner & Kaley, 1976).

The prostaglandin- and histamine- releasing actions of dextran

Dextran is known to release histamine from mast cells in rats (Beraldo, Dias da Silva and Lemos Fernandes, 1962; Baxter, 1972) and probably 5-HT and other vasoactive agents (Rowley & Benditt, 1956; Baxter, Beaven & Horakova, 1974). Dextran can also release histamine from rabbit blood (Haining, 1956). In man concentrations of 1.3 to 5.0 ng/ml of histamine have been reported as a regular occurrence after dextran infusion (Lorenz, Doenicke, Messmer, Reimann, Thermann, Lahn, Berr, Schmal, Dormann, Regenfuss and Hamelmann, 1976), probably unrelated to hypersensitivity.

Hyman, Mathé, Spannhake & Kadowitz (1976), whilst studying the effects of infused arachidonic acid on dog lungs, noted that prostaglandin synthesis from AA was greatly enhanced in dextran -, compared with blood-perfused lungs, as evidenced by a tripling of the pulmonary vascular effects of arachidonic acid. Thus dextran increases PG synthetase activity, and this is confirmed in intact dog lung lobes by Hyman, Bennett, Joiner, Chapnick, Mathé & Kadowitz (1976).

Dextran-induced anaphylactioid reactions have also been reported in man (Data & Nies, 1974; Maddi, Wyso & Zinner, 1969; Ring & Messmer, 1977) and in this situation vasoactive substances other than histamine

have been postulated to contribute to the clinical symptoms (Lorenz et al., 1976).

Experimental aims

One aim of the experiments was to develop the superfusion technique of (Vane, 1964, 1969) for detection and estimation of PG like material and other active substances such as angiotensin II and kinins (see p. 47). A technique was also developed for the extraction of prostaglandin $F_{2\alpha}$ from rapidly frozen tissue, from blood or from tracheal fluid, the concentration of which was then estimated by radioimmunoassay (see Section I, part 2, p. 25).

The experiments described in this section were carried out in order to determine whether the stimuli of raised vascular hydrostatic pressure and pulmonary oedema formation, sufficient to cause severe mechanical disturbance in lungs of intact animals and in isolated lungs could cause release of PGs into the blood, perfusate or the tracheal fluid. Possible species differences were also evaluated since many species have been used when investigating stimuli for PG synthesis.

Also studied was the ability of several perfusates to release PGLS from isolated, perfused lungs. In view of the large variety of stimuli reported to induce PG synthesis and because of the different experimental methods involved it is important to clarify if a perfusate in itself can stimulate PG synthesis.

In intact animals pulmonary vascular pressures were elevated by inflating a balloon in the left atrium of the heart. This manoeuvre is accompanied by systemic hypotension (Hauge, Bö and Aarseth, 1977) and

therefore probably by release of angiotension II. Since Angiotensin II has been reported to release PGs (p. 21) the effect of an increased blood concentration of angiotensin II on the arterial level of PGs was also studied.

PGs were detected in arterial blood of intact cats by means of continuous bioassay (Vane, 1964) of a fraction of the cardiac output. Preliminary experiments indicated release of PG-like substances (PGLS) in this preparation during pulmonary hypertension and congestion. In order to ascertain the origin of the active substances detected, bioassay was also performed on perfusate from isolated lung preparations.

The lower limit of concentration of PG detectable by bioassay is approximately 1 ng/ml of PGE_2 and $PGF_{2\alpha}$. Since the lungs actively degrade PGLS, outflow concentrations may be very low despite substantial release of PGs (Mathé & Levine, 1973), and a more sensitive assay for prostaglandin was performed. Serial radioimmunoassay of $PGF_{2\alpha}$ was made on samples of perfusate and tracheal fluid from several isolated perfused lungs (section 1, part 2, p. 25). In one pair of lungs a pulmonary metabolite of $PGF_{2\alpha}$, 15-keto, 13-14 dihydro prostaglandin $F_{2\alpha}$ was also estimated.

If pulmonary hypertension and/or oedema stimulate PG-synthesis in lung tissue this may have important physiological and pathophysiological consequences on lung function (Kadowitz, Joiner & Hyman, 1975). When released into the bloodstream PGs may also affect the systemic circulation (Malik and McGiff, 1976).

Reports of this work have been published (Scott, Vaage and Wiberg, 1977, 1979 ; Scott, Vaage & Wiberg, in prep; Wiberg, Vaage & Scott in prep . Wiberg, Scott & Vaage, 1976, in press, in press; Vaage, Scott & Wiberg, 1977, 1978; Vaage, Wiberg & Scott, in press).

Section 1 - part 2.

Radioimmunoassay estimation of $PGF_{2\alpha}$ in perfusate, lung tissue and tracheal foam from isolated lungs during pulmonary oedema

Introduction

The extent to which an unlabled compound competes with its radioactive equivalent for a limited number of receptor sites on a macromolecule is the basis for the radioimmunoassay (RIA) technique of Berson & Yalow (1960). RIA is a simple, specific and sensitive method used to study the biosynthesis and metabolism of prostaglandins. When the receptor molecule and labled PG are available, the assay is relatively simple to perform. In principle, mixtures containing labled PG, receptor molecule and the sample of PG to be quantified are incubated. Free labled PG is separated from receptor bound labled PG and the extent of binding can be determined.

Antibodies are proteins (found in the globulin fractions of blood) that are produced by vertebrates in response to the presence of an antigen, i.e. a substance that is recognised as foreign by the host. Antibodies very selectively bind the antigen that stimulates their production and this is the basis for the radioimmunoassay.

Macromolecules (proteins, polysaccharides etc.) elicit this immune response when injected, although low molecular weight compounds (haptens) such as PGs must be bound co-valently to an antigenic macromolecule (e.g. protein).

The first successful production of antibodies to certain prostaglandins was reported by Levine & Van Vunakis (1970). During the last few years the radioimmunological detection of prostaglandins at the pico gram level has been made possible by the development of antiprostaglandin sera with sufficient binding affinities.

Radioimmunassay has advantages over other methods of measuring prostaglandins. Superfusion bioassay, although a good measure of total biological activity where PG synthesis is stimulated, is neither specific nor sensitive enough to enable identification of a particular compound, nor to quantify it below the nanogram level. Chromatographic methods (including thin layer and gas-liquid chromatography) are widely used. Gas-liquid chromatography, and mass spectrometry combined are sensitive to the picomole level (Green, 1973), but samples must be extracted to prevent interference with the assays.

RIA is equally, if not more sensitive than these methods and the antigen-antibody specificity may, in some cases, allow direct analysis without previous separation from other compounds.

Increased hydrostatic pressure and pulmonary oedema in intact anaesthetized cats causes release of small amounts of PG-like substances, probably from lungs (results:section 1, p.73). However, release of PGLS is not detectable from isolated perfused lungs when measured by bioassay of the perfusate, and a more sensitive method for detection of PGs was required, since they may act as local hormones (Silver & Smith, 1975), being released into the circulation only in very low concentrations. Besides lungs have a high degrading capacity for PGs (Ferreira & Vane 1967a) which may further

decrease the amount of PGs released, with a proportional increase in metabolites in the outflow.

Bioassay tissues register short changes in PG concentration in the superfusate; smaller, more gradual, long-lasting release, as might occur in oedema formation, may go undetected as a change in tissue baseline. Pulmonary oedema, with fluid filtration into the interstitium may cause release of PGs into the interstitial fluid, perhaps remote from the site of PG degredation in endothelial cells. Alternatively it may stimulate increased tissue content of PGs. Thus the concentration of PG in tracheal fluid (a measure of interstitial PG content) and in lung tissue and plasma before and after induced oedema was determined. Lungs were perfused with plasma as described until a period of stable weight was obtained, when left atrial pressure (P_{LA}) was raised by clamping the outflow tubing.

For details of the perfusion method see p. 40 .

A report of this work has been published by Wiberg, Vaage, Scott, Teig & Gautvik, 1976.

Section 2. Release of PGLS during intravascular platelet aggregation in intact cats

Introduction

Pulmonary microembolism with platelet aggregates is probably a causal factor in the development of acute respiratory insufficiency after various types of shock and trauma (Blaisdell, 1974, Wardle, 1974), Berman, 1975), and experimentally induced platelet aggregation has been shown to cause severe pulmonary insufficiency (Vaage, 1976). There is also evidence that platelet aggregation and the subsequent release of bioactive substances cause the acute pulmonary and cardiovascular effects seen after various types of lung embolization (Thomas, Tanabe , Khan & Stein, 1965; Bø, Hognestad and Vaage, 1974).

When intravascular platelet aggregation is induced experimentally platelet aggregates are trapped in the pulmonary microcirculation and cause smooth muscle constriction in lung vessels and airways (Rådegran, 1971; Bø and Hognestad, 1972; Vaage, Bø and Hognestad, 1974; Vaage and Hauge, 1977). The cause of these lung reactions is not fully understood. However, there is considerable indirect evidence that the release of bioactive substances from platelets is important: inhibitors of the platelet release reaction also inhibit the lung effects of platelet aggregation caused by i.v. infusions of thrombin, protamine (Rådegran, 1971) and ADP (Kobayashi and Didisheim, 1973). Besides, it has been shown that after several i.v. infusions of ADP the lung effects of ADP-induced platelet aggregation eventually vanish, but are restored on infusion of a more powerful inducer of

the platelet release reaction, such as collagen (Vaage, Bø and Hognestad 1974).

However when the platelet release reaction was monitored by measuring the appearance in blood of one of the substances released, platelet factor 4 (PF-4) there was no correlation between the lung responses and the release of PF-4 after several consecutive episodes of collagen-induced intravascular platelet aggregation (Vaage and Gjesdal, 1976). These authors showed that the plasma level of PF-4 increased only after the initial collagen infusion, although lung responses were repeatedly elicited. This could mean that pulmonary smooth muscle constriction is not dependent on platelet release, or rather, that the release of smooth muscle contracting substances from platelets does not parallel the release of PF-4.

It was therefore of interest to monitor other substances released from platelets during in vivo aggregation. PGs are of special interest in this context since PG-synthesis in platelets plays a key role in the release of storage organelles (Willis, Vane, Kuhn, Scott & Petrin , 1974). Besides, both "native" PGs (Kadowitz, Joiner and Hyman, 1975) and intermediates in PG-synthesis (Palmer, Piper and Vane, 1973, Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975) are potent constrictors of pulmonary vascular and bronchial smooth muscle (see p. 167). Prostaglandins are also released when lung reactions occur during platelet agregation both in isolated, perfused lungs (Vaage and Piper, 1975) and in intact animals (Rådegran, Olsson, Alme & Granström, 1977).

The purpose of the present experiments was to study the relation-

ship between smooth muscle constriction in the lungs and the release of PGLS during collagen-induced platelet aggregation in cats.

This work has previously been reported by Vaage, Scott & Wiberg, 1976 and Vaage, Wiberg & Scott, 1978.

Section 3 : Hydrostatic Pressure Oedema and changes in C_L.

Introduction

General Introduction:

Vischer, Haddy and Stephens (1956) have defined pulmonary oedema as the pathological state in which the extravascular water content in the lung is abnormal. This is essentially the same definition as that of Laennec in 1819, who wrote that pulmonary oedema is " an infiltration of serum into the pulmonary tissue, carried to a degree such that it significantly diminishes its permeability to air."

Although this is a correct clinical definition, it overemphasizes the final event of alveolar flooding, when gas exchange is impaired (Williams, 1953; Forster, 1957; Said, Longacher, Davis, Banerjee, Davis and Wooddell, 1964). Little or no consideration is given to the earlier events in oedema formation: accumulation of interstitial oedema without significant impairment of gas exchange.

Mechanics of pulmonary oedema: the fluid transport equation

In all organs there is a net flow of fluid and protein from the vascular bed through the interstitium to the lymphatics and thence back to the the vascular system. The general equation applicable under steady state conditions is the Starling equation (Starling, 1896):

$$Qf = Kf \ (Pmv-Ppmv) - \sigma f \ (\Pi mv - \Pi pmv)$$

Qf is the net transvascular fluid flow; Kf is the fluid conductance (filtration coefficient); Pmv and Ppmv are the hydrostatic pressures (P) in the microvascular lumen (mv) and in the perimicrovascular

interstitial tissue(pmv); σ is the reflection coefficient (i.e. the effectiveness of the membrane in preventing the flow of solute compared to the flow of water); IImv and IIpmv are the osmotic pressures (II) contributed by the solutes in the microvascular lumen (mv) and the perimicrovascular interstitial space (pmv). In pulmonary oedema the large plasma proteins are the only substances that do not equilibrate across the microvascular membranes.(Visscher, Haddy and Stephens, 1956).

Alteration of one or more of these factors can upset the balance of liquid and solute exchange and lead to oedema formation. Thus according to the Starling hypothesis for bulk capillary water transport, there are several factors that could lead to pulmonary oedema: elevated capillary hydrostatic pressure, increased capillary permeability to plasma proteins, increased capillary surface area, decreased plasma colloid osmotic pressure, increased alveolar surface tension and decreased lymphatic drainage (Greene, 1965). Since one or more of these factors may change, and since they interact in a complex manner they will not be considered in detail here except in relation to the present experiments. The left atrial pressure is the main intravascular force promoting fluid and solute exit from the capillaries, and colloid osmotic pressure is the main inward force.

Extravascular forces:

Net fluid transport is affected by the pericapillary pressures of the pulmonary interstitium. This has been estimated by Meyer, Meyer and Guyton (1968) as ranging from -7 to-16 mmHg in the

normal lung. However, the role of the extravascular forces (Ppmv and IIpmv) in the progression of pulmonary oedema has long been disputed (Guyton and Lindsey, 1959; Guyton 1965; Levine, Mellins, Senior and Fishman, 1967 ; Meyer et al 1968; Wiederhielm, 1968). This is probably because they are very difficult or impossible to measure in some situations, or overridden by other factors. The role of the interstitial protein osmotic pressure has been researched by Staub's group (Staub, 1970; Staub, 1971).

In summary, they have shown that every increment in microvascular hydrostatic pressure is half offset by an increase in the protein osmotic pressure difference. Thus, changes in IIpmv and increases in lymph flow are major factors in keeping the lung "dry".

Membrane parameters:

When membrane permeability changes (i.e. Kf and σ are altered) fluid filtration increases without necessarily changing hydrostatic pressure. Clinically this is characterised as non-cardiogenic pulmonary oedema (Robin, Carey, Grevnik, Glauser and Gaudio, 1971). Experimentally, alloxan can induce this (Aviado, 1953; Hultgren and Flamm, 1969). There is no increase in pulmonary blood volume as measured by the content of P^{32}-marked erythrocytes (Aviado, 1953). Microscopic studies show that alloxan destroys pulmonary capillary cells and the underlying basement membrane, resulting in severe injury to the capillaries. Increased lung

weight and extravascular water are readily demonstrated on post-mortem examination, but impaired perfusion makes it difficult to document by the indicator dilution technique.

EDTA, a chelator of calcium and magnesium ions, induces permeability oedema in the isolated, perfused rabbit lung (Nicolaysen 1971b). However, no abnormalities in the ultrastructure of the microvascular membrane were observed (Hovig, Nicolaysen and Nicolaysen, 1971). Some electron microscopy studies have shown a complete breakdown of membrane structure in permeability oedema (Schulz, 1959; Cottrell, Levine, Senior, Weiber, Spiro & Fishman, 1967). That is, the endothelium no longer acts as a selective barrier to protein permeability, and fulminant oedema develops (Robin et al, 1971).

One characteristic of increased permeability oedema is that the protein flow (lymph flow times concentration of lymph protein) is always increased (Brigham, Woolverton and Staub, 1973).

Intravascular forces:

Elevated intravascular hydrostatic pressure (Pmv) is the cause of probably the commonest clinically observed form of pulmonary oedema, congestive heart failure. It is due to an abnormality in heart function, especially left ventricular failure or mitral stenosis. Non-cardiogenic causes include pulmonary venous-occlusive disease, pulmonary venous fibrosis or stenosis or overinfusion of physiological solutions (Robin, Cross and Zelis, 1973).

Although the initiating stimulus for lung water accumulation varies, the clinical manifestations are the same for all kinds of lung oedema : diffuse X-ray picture and progressive impairment of

gas exchange and mechanics. In addition, the sequence of fluid accumulation is essentially the same. This has been shown by Staub, Nagano and Pearce (1967). Microscopic examination of rapidly-frozen lungs showed that the earliest accumulation of fluid is in the interstitial connective tissue compartment around larger blood vessels and airways. Alveolar walls then became thickened and finally, when the interstitial compartment was filled, alveolar flooding took place. In addition, they showed that alveolar filling occured independently and rapidly in individual alveoli, without the occurrence of air trapping, when they reached a critical configuration at which the existing transpulmonary pressure could not maintain alveolar stability. Fluid accumulates initially in the interstitial space and not in the alveoli, probably because the capillaries are more permeable than the alveolar epithelium to water (Wangensteen, Wittmers and Johnson, 1969). Schneeberger-Keeley and Karnovsky (1968), using electron microscope techniques, have clearly shown that capillary endothelial cell junctions are looser than those of alveolar epithelium. Thus macromolecules (eg plasma proteins and haemoglobin) can cross endothelium at lower driving pressures than are required for epithelium (Pietra, Szidon, Leventhal & Fishman, 1969). However, the presence of protein in oedema fluid shows that a large change in permeability must have occurred. Hovig et al.(1971) did not show any alveolar disruption in high pressure oedema, although probably intracallular junctions must have opened.

Hydrostatic oedema and changes in lung compliance

As early as 1887, von Basch produced acute pulmonary vascular engorgement in living dogs and observed changes in the mechanics of the lungs. In open-chested preparations, ventilation was produced by means of a pump with constant pressure amplitude. With congestion the tidal volume decreased and end expiratory lung volume increased simultaneously. The reduction in tidal volume was regarded as increased lung stiffness, i.e. reduced specific compliance, and the increased end expiratory volume (functional residual capacity, FRC) as evidence of vascular engorgement.

Other animal studies confirmed von Basch's work. Drinker, Peabody and Blumgart (1922) showed that occlusion of cat pulmonary veins was associated with a reduction in pulmonary ventilation, and that these changes were immediately reversed when vasoconstriction was removed. Mack, Grossman and Katz (1947) found decreases in lung distensibility following acute vascular engorgement in excised dog lungs and in lungs of intact dogs. Also Heyer, Holman and Shires (1948) found marked reduction in lung distensibility following rapid infusion of saline solution. The lungs of these animals were found post mortem to be haemorrhagic and heavy, and it is not possible to dissociate the possible contribution of alveolar oedema from their results.

Borst, Berglund, Whittenberger, Mead, McGregor and Collier (1957) have separately examined in vivo the mechanical effects of changes in pulmonary blood flow and pulmonary arterial pressure from those of left atrial pressure elevations. Large variations

in pulmonary blood flow did not influence the mechanical behaviour of the lungs. However, elevation of left atrial pressure caused a reduction in dynamic lung compliance (C_L) of about 20-30%, roughly proportional to the magnitude of P_{LA} elevation. C_L returned to control level within 10 sec of lowering P_{LA} from 50 to 6 cmH_2O. This would argue against increased filtration causing the fall in C_L as this would have to be extremely rapid, and could rather be due to distension of veins or capillaries. These authors found reductions in C_L of smaller magnitude than those reported by Bondurant, Hickam and Isley (1957) after acute central congestion in normal human subjects, due to inflation of an anti-gravity suit or to submersion in water. They showed more than a 50% reduction in C_L at P_{LA} not greater than 45 cmH_2O. This may represent a true difference between dog and human lungs, or may be due to differences in techniques of measurement.

The changes in C_L observed in the experiments of Borst et al (1957) are small compared to those seen in patients with chronic congestive heart failure or pulmonary hypertension. Possibly changes in lung mechanics in such patients are largely caused by factors other than the pulmonary congestion per se, such as chronic changes in the vessel walls and parenchyma or increased heart size.

Experimental work in many species supports the concept that oedema formation in the lung begins in the interstitial perivascular tissue while the escape of fluid into the alveoli takes place at a later stage (see general introduction to this section).

However. what is not clear is whether the fall in compliance which these workers measured was due to distension of the intravascular compartment of the lungs as they have shown or, as some other authors claim,to pulmonary interstitial oedema (Gump, Zikria and Mashima, 1972; Sladen, Laver and Pontoppidan, 1968; Rief, McCurdy, Coalson and Greenfield, 1972).

Gump, Zikria and Mashima (1972) confirm the observations of the sequence of fluid accumulation in the lungs. However, they conclude that C_L and, to a lesser extent, pulmonary shunt flow are sensitive indicators of interstitial oedema. They ensured that alveolar oedema had not occurred (by X-rays and histological examination) when they created interstitial oedema by steam inhalation in intact dogs. In addition, Sladen, Laver and Pontoppidan (1968) found a 30% fall in lung compliance when extravascular lung water increased in respiratory failure. They suggested using the measurement of compliance as a means of detecting oedema in its early (interstitial) stage. Also, in experimental studies in perfused dog lungs, Rief et al.(1972) correlate their observed decrease in C_L with interstitial oedema since it was not reversible on releasing the venous constriction. Electron micrographs showed that very little alveolar oedema had occurred, but that interstitial oedema was clearly visible in the perivascular space.

On the other hand, Hauge, Bo and Waaler (1975) have concluded that C_L falls in association with changes in pulmonary blood volume. They clearly showed that a sharp fall in dynamic C_L (about 20%) took place when pulmonary blood

volume (PBV) increased in hydrostatic oedema in isolated rabbit lungs. Also, returning P_{LA} to control levels restored C_L in several seconds, even when extravascular fluid volume had almost tripled (Bo, Hauge, Nicolaysen and Waaler, 1973). Hauge, Bo & Waaler (1975) have also shown that, after the initial reduction due to increased P_{LA}, C_L then remained constant, though reduced, during the period of raised P_{LA} until a second, more gradual fall occurred. The question then arose as to whether the period of stable, reduced C_L could be equated with interstitial oedema formation and whether this secondary fall, which was irreversible on returning P_{LA} to control (own experimental observations) coincided with the start of alveolar flooding in this preparation.

The purpose of this study was to perform a detailed histological examination to see the sequence of fluid accumulation, and to investigate a possible correlation between the onset of alveolar oedema and the secondary fall in C_L. This project (unpublished) was done in collaboration with Drs. Nicolaysen and Hauge.

SECTION 1. THE RELEASE OF PGs FROM LUNGS DURING INCREASED HYDROSTATIC PRESSURE AND OEDEMA

METHODS

Anaesthesia:

Isolated Lung Donors

Rabbits weighing 2.5-4.0 kg were used. Fifteen rabbits were premedicated with 10mg diazepam (Valium) intraperitoneally. All rabbits were anaesthetized with 30-40 mg/kg sodium pentobarbitone (Nembutal ® diluted 1:3 with isotonic saline) into the v. auricularis caudalis. Cats weighing 2.0-2.5 kg and guinea-pigs (320-800g) were anaesthetized with 30-40 mg/kg Nembutal ® intraperitoneally.

Intact Cats

Cats weighing 3.3-4.4 kg were used for the experimental procedure. Cats weighing 1.8-2.3 kg were used as blood donors. All cats were anaesthetized with 30-40 mg/kg Nembutal ® ip. Blood donors were heparinized with 500 IU/kg heparin and exsanguinated by cardiac puncture. One thousand IU heparin powder was added per 100 ml of this blood.

The Isolated Lung Preparation

This lung preparation is according to the method of Hauge, Lunde and Waaler (1966). Animals were tracheotomized and ventilated at constant volume with positive pressure at approximately 7 cm H_2O peak inspiratory pressure (P_{PI}) and 1-2 cm H_2O end expiratory pressure (P_{EE}). Lignocaine (1%) was injected (3 ml total dose) subcutaneously at intervals along the sternum and the thorax was opened widely by a

midline incision and the pleura were dissected open. The pleural structures above the diaphragm and the phrenic nerves were cut. The oesophagus was ligated with two threads and cleaned as far as possible of pleural structure before being cut close to the diaphragm between the two threads. Two threads were placed around the inferior vena cava but not tied.

The pericardium was removed to expose the heart. The thymus was dissected free, ligated close to the trachea and removed. Tissue over the trachea was dissected until the trachea was exposed as far as possible and the oesophagus was clamped at the level of the tracheal cannula and drawn carefully out. Thread was placed around the aorta and the pulmonary artery close to the heart.

Heparin (10 mg in 1 ml of saline solution to rabbits and cats; 1.5 mg to guinea-pigs) was injected directly into the right ventricle. The inferior but not the superior vena cava was tied and ventilation was stopped. The preparation (trachea, lungs and heart) was removed, being careful not to touch or in any way to damage the lungs, by lifting the tracheal cannula and dissecting carefully close to the dorsal surface of the thoracic cage and cutting the descending aorta.

The preparation was then suspended by a clamp on the apex of the heart ventricles and a plastic cannula (stainless steel cannula for guinea-pigs) was placed in the pulmonary artery (PA) by insertion through the wall of the right ventricle. The cannula was tied with the ligature which had been placed round the PA, at the same time ligating the aorta, and secured by a thread tied round the ventricles of the heart. The cannula was primed with saline. A wide necked curved glass (or stainless steel) outflow cannula was placed in the left atrium, by means of an incision in the wall and securely tied.

The cannula also curved outwards at the rim which enabled it to be drawn out as far as possible to minimise resistance. The width of the cannula also minimised resistance to flow. The preparation was suspended freely in a closed, heated Perspex chamber from the thread tied round the heart and connected to a force transducer (Sanborn FTA 100-1) (Nicolaysen, 1971a; also see fig. 2).

Perfusion

Lung perfusion was always begun a maximum of 10-15 min after the animals' circulation was stopped. Perfusion was begun with Krebs Ringer solution and the first 50-100 ml used to wash out residual blood from the preparation was discarded. The change from Krebs Ringer to the required perfusate was performed within 1-2 min of the start of perfusion.

The lungs were perfused by a variable speed peristaltic pump in a recirculating system with constant volume inflow of between 100 to 380 ml/min for cat or rabbit lungs and 20-25 ml/min for guinea-pig lungs. Pump rate was set to give a perfusion pressure in the pulmonary artery (P_{PA}) of between 10 and 15 mm Hg. P_{PA} was measured from a sidearm of the inflow cannula which was positioned at the level of the hilum, and recorded on a Hewlett-Packard recorder (p.188).

Perfusate temperature was thermostatically controlled at 37°C in water jackets round the venous reservoir, the Perspex chamber enclosing the lungs and the inflow tubing to the lungs. Left atrial pressure was kept constant during control perfusion at 1 mm Hg. The total volume of the perfusion system was about 300-350 ml unless otherwise stated.

Fig. 2. Diagram of the isolated lung perfusion apparatus.

Ventilated lungs were perfused in a recirculating system. Perfusion pressure was measured in the pulmonary artery (P_{PA}). A fraction (10ml/min) of the outflow from the pulmonary veins (pulm vein) was pumped over the assay tissues: rat stomach strip (RSS), rat colon (RC) and chick rectum (CR) and returned to the venous reservoir (Reservoir). Lungs were suspended from a weight transducer and enclosed in a plastic box surrounded by a water jacket, thermostatically-controlled at 37°C. Perfusate was also heated to 37°C in the venous reservoir and before superfusing the assay tissues. Left atrial pressure could be raised by clamping successive steps of a ladder of tubes on the venous side of the lungs.

Fig. 2.

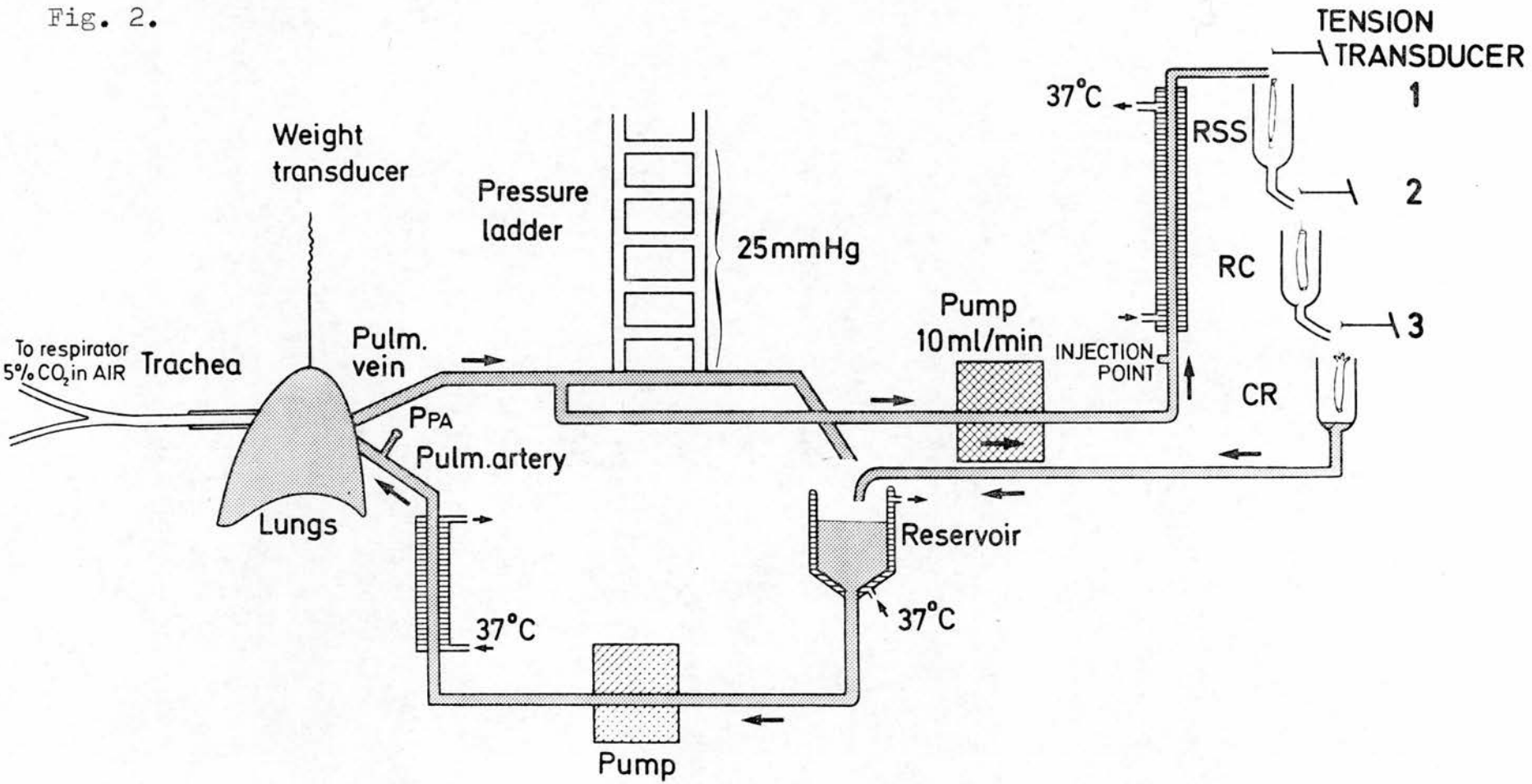

TENSION
TRANSDUCER
1
2
3
37°C
RSS
RC
CR
Weight
transducer
Pressure
ladder
25mmHg
Pump
10ml/min
INJECTION
POINT
To respirator
5% CO_2 in AIR
Trachea
Pulm.
vein
P_{PA}
Pulm.artery
Lungs
Reservoir
37°C
37°C
Pump

Fig. 2 shows a diagram of the perfusion system.

Ventilation

Isolated rabbit and cat lungs were inflated after the start of perfusion to about 20cm H_2O for about 3-5 sec. They were ventilated at positive pressure using a Starling "Ideal" pump (Palmer Ltd, London) at a frequency of 20 strokes per min, and a ventilation volume of about 40-50 ml.

Ventilation was begun at constant pressure: peak inspiratory pressure was set at 7-10cm H_2O by means of a water seal overflow system which could be adjusted. Pump volume was set such that P_{TP} was between 5 and 9cm H_2O and ventilation at constant volume commenced. P_{EE} was maintained at 1-2cm H_2O throughout all experiments by means of a water seal. Ventilation gas was 5% CO_2 in air and perfusate pH was 7.4-7.5 throughout each experiment.

P_{TP} was measured with a differential transducer and monitored on a Hewlett-Packard 2-channel recorder (7402A). In experiments where bioassay of prostaglandins in perfusate from isolated lungs was performed, P_{TP} was measured with a Grass pressure transducer and monitored on an 8-channel Grass polygraph, model 7B.

Guinea-pig lungs were not ventilated because of high airway resistance. However, the perfusate was gassed with 5% CO_2 in air.

Measurement of Weight Changes

Lungs were freely suspended from the ligature tied around the ventricles of the heart and changes in preparation weight were recorded. From the start of perfusion, the weight changes followed a pattern of 1) a period of gradually decreasing weight, which may last up to 30 min

but was normally shorter.

This is followed by 2) a period of stable weight and 3) a period of increasing weight which accelerated until eventually oedema occurred. The length of these periods varies according to the type of perfusate used (Nicolaysen, 1971a). When KR was used as perfusate, the period of decreasing and stable weight was short (approx. 15 min) and lungs quickly became oedematous thereafter.

In experiments where left atrial pressure (P_{LA}) was raised the lungs were perfused at P_{LA}=1 until the period of stable weight was obtained. P_{LA} was raised by clamping steps of a ladder of tubes on the venous side of the lungs which diverted the outflow perfusate upwards before it returned to the venous reservoir (see fig. 2). Pressure could thus be raised in increments of 5 mmHg. When P_{LA} is raised there is an initial steep increase in preparation weight followed by a more gradual, steady weight gain (fig. 9). The first phase is due to increased pulmonary blood volume due to vasodilatation and opening of more capillaries (Lunde and Waaler, 1969). The second phase is due to accumulation of fluid in the extravascular space (Nicolaysen, Aarseth and Waaler, 1976). Weight increases were measured 30 sec after P_{LA} was raised, thus reducing the contribution to the weight increase of raised pulmonary blood volume .
The change in weight after 30 sec was measured in order to be able to estimate the extent to which extravascular fluid had increased in the preparation, distending the interstitium and causing alveolar oedema.

When P_{LA} was raised there was a simultaneous increase in P_{PA}, and in P_{TP} indicating a reduction in dynamic lung compliance (C_L; Hauge, Bö and Waaler, 1975; see Section 3.).

Bioassay

To measure prostaglandin-like substances (PGLS) in blood or lung perfusate a modification of the superfusion technique of Gaddum (1953) as developed by Vane (1964, 1969) was used. This is an on-line bioassay in which organs are superfused with a stream of blood (intact animals) or perfusate (isolated organs).

Perfusate was assayed for its PG content by superfusion over a series of smooth muscle tissues and returned intravenously (fig. 2). The choice of assay tissues depends on the substance to be estimated. The assay tissue chosen must be sensitive to the particular substance to be measured and relatively insensitive to other substances in the bathing fluid. The combination RSS, RC and CR was chosen for their sensitivity to PGs. Sometimes a RbA was included to detect PG precursors (p.47).

The specificity of the bioassay is increased by the use of antagonists. The assay tissues rabbit aorta (RbA), rat stomach strip (RSS), rat colon (RC) and chick rectum (CR) were bathed for at least 1 hr before the start of the experiment in Krebs Ringer (KR) solution (see Appendix p.189) gassed with 95% O_2 and 5% CO_2. To this solution was added 5 g/l of glucose and a mixture of antagonists of ACh (hyoscine hydrobromide 0.15 mg/l), catecholamines (phenoxybenzamine 0.14 mg/l and propranolol hydrochloride 2.28 mg/l), 5-hydroxy tryptamine (methisergide bimaleate 0.2 mg/l) histamine (H_1 receptor blocker, mepyramine maleate 0.14 mg/l) and glucose (5 g/l) (Piper and Vane, 1971). The blocker solution also usually contained indomethacin 5 ng/l to prevent the intramural generation of prostaglandins (Vane, 1973).

Rat stomach strip (RSS), rat colon (RC) and chick rectum (CR) contract differentially to PGE_2 and $PGF_{2\alpha}$: RSS and CR are more

sensitive to PGE_2 than the RC, while RC and RSS contract to $PGF_{2\alpha}$ (Palmer, Piper and Vane, 1973 ; Said, 1974).

However, these three assay tissues are sensitive to other substances such as intermediates and metabolites in the conversion of arachidonic acid, and to vasoactive substances against which they are not blocked such as kinins and angiotensin II. When assaying substances in tissue superfusate the effects on the tissues must be **compared** with calibrating doses of that substance. However, when the assay system is used for detection of unknown substances, tissue patterns may be **compared** with calibrations but the contributing effects of other active substances (if no suitable blocker exists for them) to tissue activity must be considered.

The Rat Stomach Strip (RSS)

RSS is sensitive to PGE_2, $PGF_{2\alpha}$, the PG endoperoxides and thromboxane A_2 (TxA_2) (see p.150). The PG metabolites also retain some activity (p.151).

The Rat Colon (RC)

The rat colon contracts to $PGF_{2\alpha}$ (Regoli and Vane, 1966) and is also slightly sensitive to PGE_2 (Said, 1974).

The Chick Rectum (CR)

The chick rectum is sensitive to PGE_2, $PGF_{2\alpha}$ and, to a lesser extent, to endoperoxides and TxA_2 (Bunting, Moncada and Vane, 1976) Tissues cannot be blocked against TxA_2 or PG endoperoxides.

The Rabbit Aorta (RbA)

In several experiments a helically-cut strip of rabbit aorta was included. The "rabbit aorta-contracting substance" (RCS; Palmer, Piper and Vane, 1973), which is often released when PG synthesis is stimulated, has been discovered to consist of TxA_2 with small amounts of the PG endoperoxides (Hamberg, Svensson and Samuelsson ,

1974). RbA is very sensitive to TxA_2 and less sensitive to the PG endoperoxides (PGG_2 and PGH_2), but is insensitive to PGE_2 and $PGF_{2\alpha}$ (Hamburg, et al 1975; Bunting, Moncada and Vane, 1976).

Thus the bioassay method gives a good qualitative measure of biological activity in the convertion of arachidonic acid (AA; see fig. 1) although quantitive identification of each compound is difficult, especially since synthesis and release probably involves a mixture of substances.

Effects of Non-Prostaglandin Substances on the Bioassay Tissues

Recently Said and Mutt have reported release of vasoactive and spasmogenic lung peptides. These were found to contract RSS and RC (Said and Mutt, 1977.) The rat colon contracts strongly to Angiotensin II (Regoli and Vane, 1964) but is much less sensitive to other vasoactive substances such as 5HT and bradykinin. Cat jeunum (CJ) is sensitive to bradykinin (Ferreira and Vane, 1967b) and contracts to this substance.

The Assay System

A thread from the tissues was tied to a transducer which was positioned immediately above the tissue bath. The tissue was suspended in the plastic bath to protect it from temperature changes and the lower thread was kept in place by a close-fitting plastic spout placed on the base of the bath. This anchored the tissue in place and the tissue tension could be altered manually by drawing the thread out or by moving the vertical position of the bath. Isometric tension of the tissues was measured by transducers (see figs. 3 and 4 and p.49), and recorded on a Grass polygraph (model 7B). Tissue superfusate was pumped through

Fig. 3. The electronic design of the force transducers is shown. The semiconductor transducer element consists of a single crystal silicone beam (transducer beam), having one diffused resistor (R) on each side. When the tip of the beam is deflected (indicated by the arrow to the right), the zones into which the resistors are diffused will be stressed and their resistance changed. The resistor in the compressed zone will decrease its value and vice versa. The "active"resistors in the transducer beam were connected to two "passive" (constant) resistors (R) to form a complete Wheatstone bridge. U is the power supply.

Fig. 3.

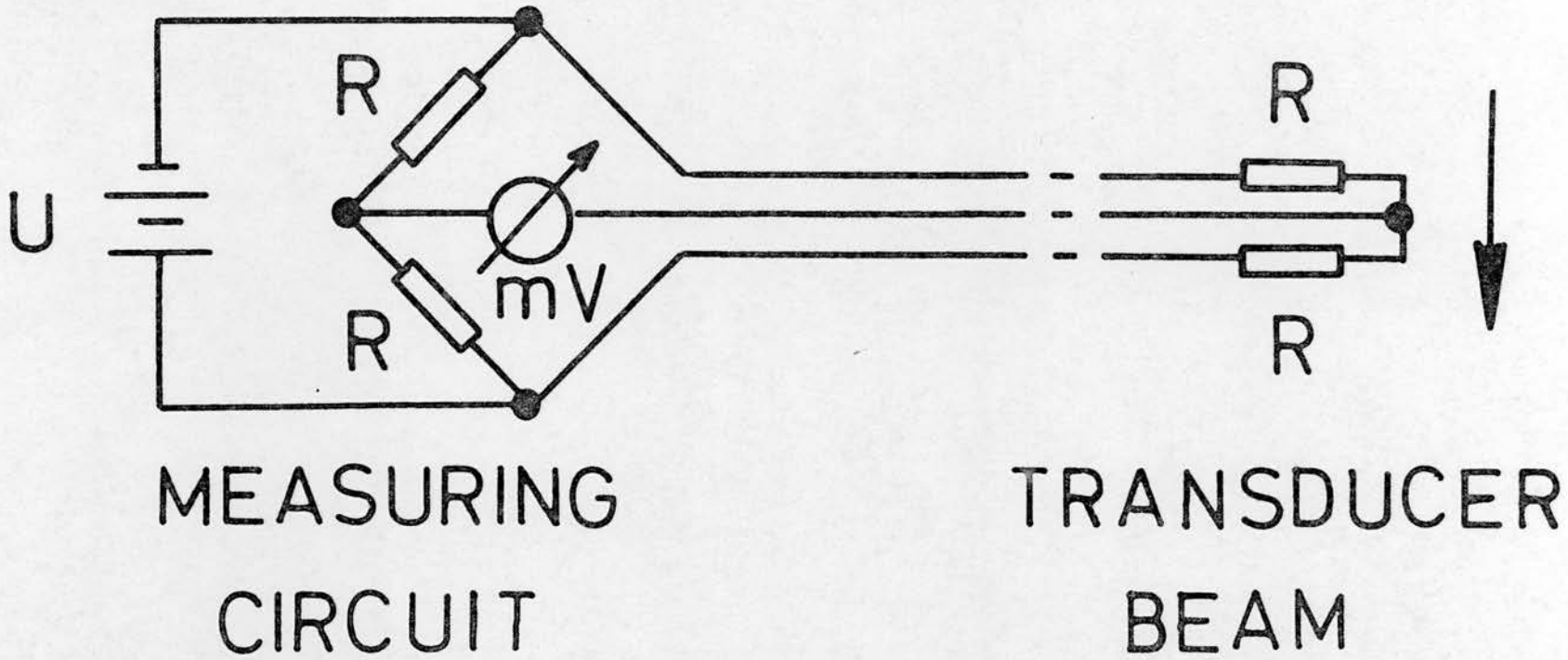


Fig. 4. The mechanical design of the force transducers is shown schematically. The force to be measured displaced a spring, as indicated by the vertical arrow on the far right. The transducer element then measured the position of the spring. Maximal deflection of the beam of the transducer element (length 3mm) was 50µm. Due to the selected rigidity of the spring this maximal deflection was obtained when a weight of 7g was hung on the spring. This corresponded to a deflection of 420 to 450 µm of the tip of the spring. Further deflection of the spring was blocked to avoid overload.

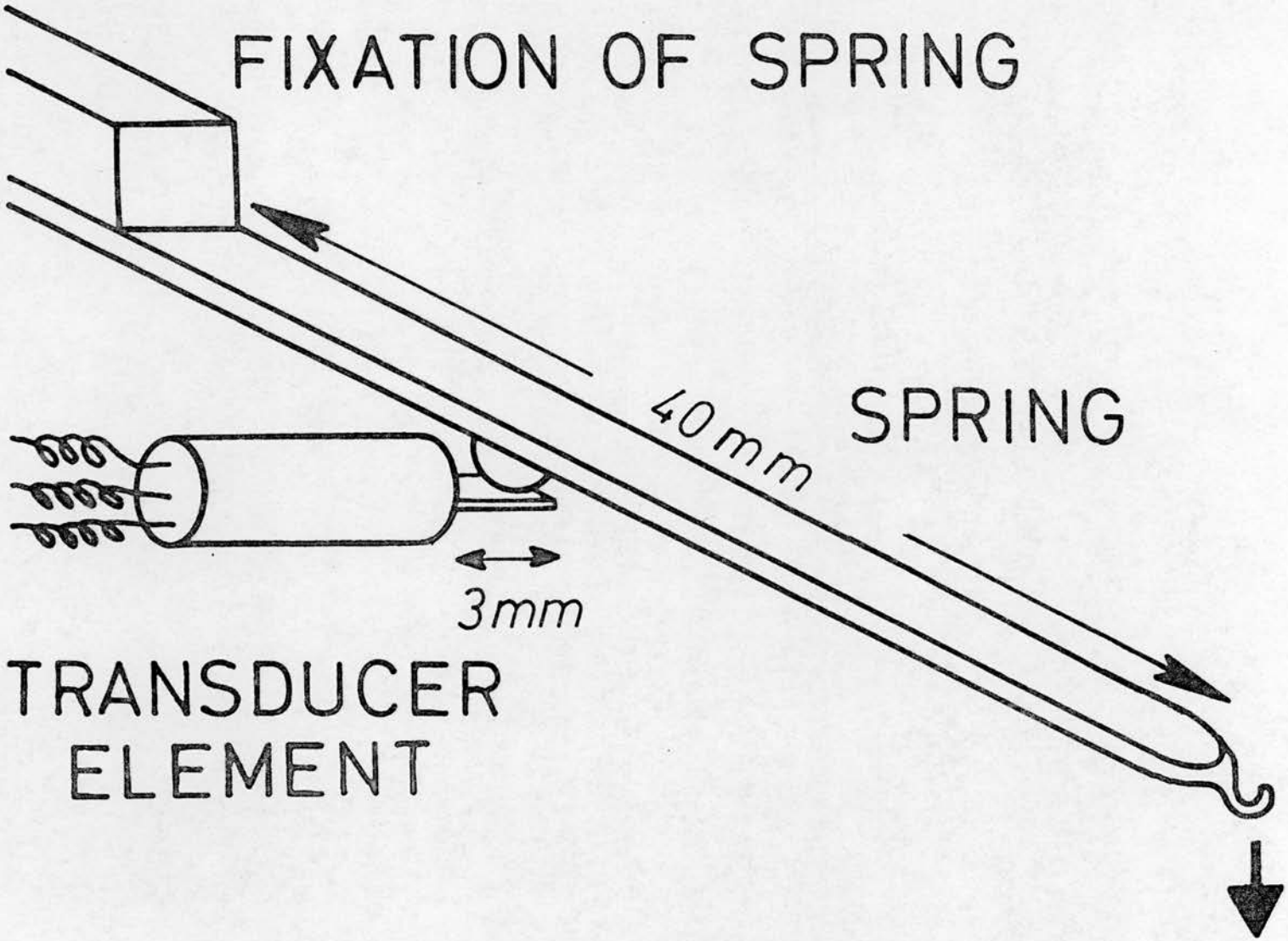


Fig. 4.

2mm diam. silastic tubing and heated (37°C) by a water jacket parallel current heat exchanger surrounding the tubing for 40 cm (see fig. 2). The tubing was arranged so that perfusate flowed down the thread which suspended the first tissue without the thread actually touching the tubing Perfusate was collected in the spout of the first tissue bath and channelled over the upper thread of the tissue beneath.

The Assay Tissues : Preparation

1) Rat Stomach Strip

Male or female Sprague-Dawley rats were either stunned by a blow on the head or lightly anaesthetized by ether and the abdomen opened. The whole stomach was removed into Krebs Ringer solution. The rat stomach strip was cut from the upper fundic part by the method of Vane (1957). Two or three parallel cuts were made on the lesser curvature and the resulting strip was about 6 cm long. The mucosa was not cleaned away. Threads were tied at either end and the tissue placed in Krebs Ringer solution (gassed with 95% O_2 and 5% CO_2) at 20°C containing the antagonist mixture (see p.46) for at least 1 hour before being placed in the cascade.

2) The Rat Colon

The ascending colon (4-5 cm long) was removed from the rat into Krebs Ringer solution and the contents of the lumen were washed out. Connective tissue and mesentery were removed and, using a needle, a thread was tied through the wall of the colon at either end, thus allowing the lumen to remain open, and the superfusate to reach and drain from the lumen.

3) The Chick Rectum

Chickens (1-2 weeks old) were killed by decapitation and the

rectum was removed from the anus to the junction with the descending colon, which has three branches. A piece of rectum approximately 2-3 cm long with the three branches (4mm) was removed. The tissue, in cold Krebs Ringer solution, was cleaned of mesentery and lumenal contents and tied at the anal end by the same method as for the rat colon. At the proximal end one branch was tied and the others left open. The tissue was placed in blocked Krebs Ringer (gassed with 95% O_2/5% CO_2) for at least one hour.

4) The Rabbit Aorta

The thoracic aorta was cut from a rabbit which had been anaesthetized with pentobarbitone (Nembutal ®) 30 mg ip. A length of approximately 1.5-2 cm was removed into Krebs Ringer solution which had been gassed with 95% O_2 and 5% CO_2. It was cleaned of mesentery and a spiral strip was cut which measured about 4-5 mm wide and 3 cm long, tied at both ends and placed in blocked Krebs Ringer solution (p.46) gassed with 95% O_2 and 5% CO_2.

In some experiments the rabbit aorta was removed from the lung donor animal and stored in Krebs Ringer solution overnight in the fridge for use in the next experiment.

5) The Cat Jejunum

The cat jejunal strip was prepared as described by Ferreira and Vane (1967b). A piece of jejunum was taken from a cat which had been anaesthetized with pentobarbitone (30-40 mg/kg ip) and was placed in cold Krebs Ringer solution which had been gassed with 5% CO_2 and 95% O_2. The tissue was cut into a longitudinal strip about 4-6 mm wide and 5 cm long and placed in Krebs Ringer containing glucose and blockers at 3°C until used (1-3 days).

The time between the perfusate leaving the lungs and reaching the

first assay tissue was 1-1.5 min. When the rabbit aorta was included it was always the first tissue in the cascade, since the half life of RCS is short (p.150). In experiments where CJ was used it was placed at the top. Otherwide the order of the assay tissues in series was always RSS, RC and CR, although no effect of changing order has been reported. According to Vane (1969) no significant release of active substances occurs from tissues themselves, nor is there modification of the substances present in blood.

Standard solutions of PGE_2 and $PGF_{2\alpha}$ were made up daily in isotonic saline from stock solutions in 100% ethanol, and calibrating doses were infused into the superfusate upstream to the assay tissues. RSS and CR were sensitive to as little as 0.5-1 ng/ml of PGE_2, while RC and RSS were sensitive to 1-2 ng/ml $PGF_{2\alpha}$. Tissues were also calibrated with angiotensin II (0.1-1.0 ng/ml) and bradykinin (1-4 ng/ml).

Upstream to the assay tissues superfusate was heated to 37°C by means of a surrounding water jacket. Tissue superfusate temperature fell during superfusion by approximately 0.5°C at each assay tissue. The tissues were mounted and superfused for approximately 2-3 hr with KR solution containing blockers before superfusion with blood or lung perfusate was begun. The basal tension of RSS, RC and CR was set at 1-2 g while that of RbA and CJ was set at 3-4 g.

The Intact Cat Preparation: Elevation of P_{LA}

Experimental animals were tracheotomized and a muscle relaxant (alloferine 0.5 mg/kg) was given. They were then ventilated with positive pressure at constant volume (frequency 24/min) by a Starling "Ideal" pump. End expiratory pressure, P_{EE}, was kept at 1.5 cmH_2O.

Arterial pH was maintained at 7.35-7.45 by adjusting the tidal volume. The thorax was opened by a sternum-splitting incision and polythene catheters placed in both femoral arteries. A catheter was placed in the pulmonary artery through the wall of the right ventricle: using surgical sutures, four stitches were made in the outer tissue of the muscle wall to form a circle about 5mm diameter and the ends tied about 5mm above the wall. A piece of fishing twine was inserted into a polythene catheter until it protruded 2mm from the end. This end was inserted into the ventricle through the centre of the suture, the fishing twine removed and the cannula was positioned in the pulmonary artery, as judged by the pressure recording, and securely tied.

Femoral arterial (P_{FA}) and pulmonary arterial (P_{PA}) pressures were recorded on a Grass polygraph (model 7B). An incision was made in the left atrium of the heart and a saline-primed balloon catheter (Folatex No. 8) was inserted for the recording of left atrial pressure (P_{LA}) and infusion of PGs. P_{LA} could be raised by inflating a 1cm long balloon which lay in the outer circumference of this catheter at the proximal end. This raised hydrostatic pressure in the pulmonary circulation.

Cardiac output (CO) minus coronary flow was measured by an electromagnetic flow probe placed round the ascending aorta. Pulsatile and mean flow were recorded. Tracheal pressure (P_{TP}= tracheal pressure in open-chested animals) was measured with a pressure transducer connected to the tracheal cannula. The signal was amplified (Sanborn 350 1000B DC preamplifier) and recorded on a Sanborn 320 dual channel DC amplifier-recorder. Polythene catheters were placed in one carotid artery and an external jugular vein for the removal and return of blood in the extracorporeal circulation.

Bioassay of PGs and Angiotensin II

Thirty minutes after completion of the surgical procedures lungs were hyperinflated (P_{TP}= 20 cmH_2O for 3-5 sec). Cats were heparinized (1000IU/kg) and blood superfusion of the bioassay tissues was begun (as described on p.46). Blood (10ml/min) was pumped from a carotid artery over the tissues and drained back under gravity into the external jugular vein. The animals were transfused with 90-200 ml donor blood and in all but 2 experiments also with 30-60 ml of dextran (Macrodex® 6%, Pharmacia, Sweden) to compensate for blood loss into the extracorporeal circulation.

When the extracorporeal circulation was started there was a small increase in peak inspiratory pressure which showed that airway constriction had occurred. This increase in peak inspiratory pressure declined gradually and hyperinflation of the lungs (P_{TP}=20 cmH_2O for 3-5 sec) returned it to its previous value. An increase in P_{PA} of 2-5 mmHg also occurred at this point in most experiments. This increase often returned by itself after 10-30 min or after a hyperinflation, but was sometime irreversible. CO fell slightly (10-15ml/min) when extracorporeal circulation began but returned on infusion of blood and dextran solution. These changes were probably due to activation by the foreign surfaces of the extracorporeal circulation of microaggregates of blood platelets (Timmes and Wilson, 1973).

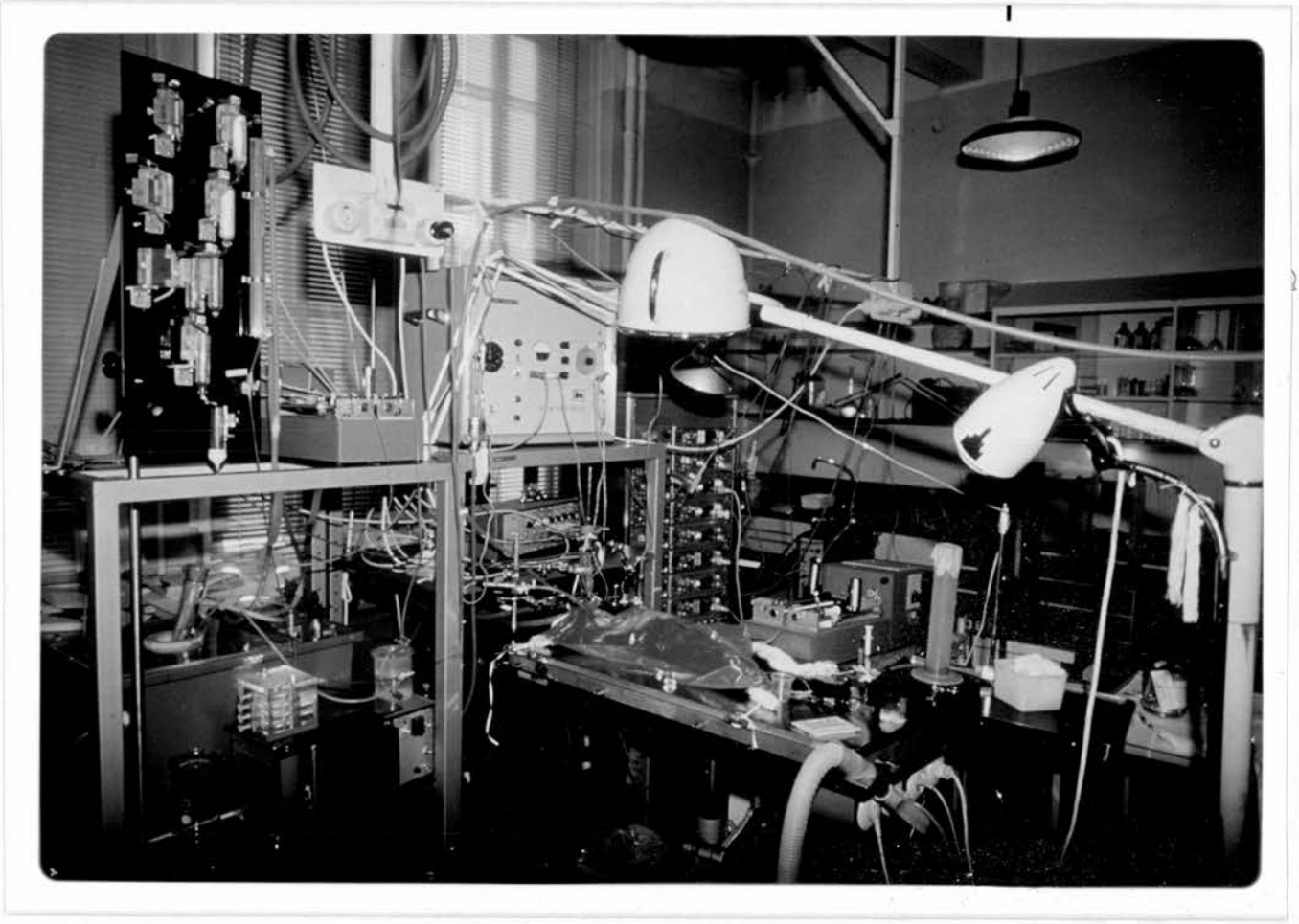
When blood reached the assay tissues they contracted (often full scale on the polygraph i.e. 4g tension) then gradually relaxed over 10-30 min. Tissue basal tensions were adjusted if required to 1-2g (RSS, RC and CR) and 3-4g (RbA and CJ). Exposure to blood decreased tissue sensitivity to calibrating doses of PGs, often by as much as 50%, and the sensitivity of the recorder was increased if required. The

spontaneous activity of the RSS and CR decreased when blood superfusion began, often returning to previous levels of spontaneous activity after about 1 hr of superfusion. Spontaneous activity of the colon usually increased during blood superfusion. Seasonal variations in the sensitivity of the bioassay tissues were noted, the tissues being more sensitive to calibrating doses of PGs in Spring and Autumn and relatively insensitive in Winter. Fig. 5 shows the experimental set up for the bioassay of PGs in blood from intact cats.

Experimental Protocol

After blood superfusion of the tissues was started, there was a time lag of approximately 30 min before the haemodynamic parameters and the tension of the assay tissues were stable. Tissues were calibrated with PGs (1-2ng/ml PGE_2 and $PGF_{2\alpha}$) and in some experiments angiotensin II (0.1-1.0ng/ml). Lungs were hyperinflated ($20cmH_2O$ for 3-5 sec) and 5 min later P_{LA} was elevated by inflating the balloon catheter and was kept high (20-50mmHg) for a maximum of 10 min or for as long as P_{FA} and CO stayed at a stable but reduced level. In some animals more than one pressure elevation was performed, with an interval between them of at least 30 min. Assay tissues were calibrated between periods of pressure elevation.

Fig. 5. The intact cat experiments.



SECTION 1, PART 2. RADIOIMMUNOASSAY ESTIMATION OF $PGF_{2\alpha}$

METHODS

In 4 lungs a series of plasma samples (5ml) were withdrawn, via a needle inserted into the outflow cannula in the left atrium, before and during the induction of pulmonary oedema. Oedema was caused by raising and maintaining P_{LA} at 25 mmHg (2 cat lungs), 20 mmHg (1 rabbit lung) and 15 mmHg (1 rabbit lung) throughout the experiment. Samples were collected on ice and stored at -20°C until the extraction procedure was begun. This was performed according to a modification of the method of Gutierrez-Cernosek, Morill & Levine, 1972 and Gautvik, Teig, Wiberg, Bronsted & Christoffersen, in prep. 2.5 ml of each plasma sample was vortexed for 15 min with 5 ml methyl alcohol:100% ethanol mixture (4:1) and left to stand for 1hr at 20°C. Tubes were centrifuged (3000 rpm) for 30 min at 20°C and the supernatants drawn off into test tubes. The precipitate was then washed with 1.25 ml of the methyl alcohol:ethanol mixture and centrifuged as before. The supernatants were combined, evaporated under nitrogen at 45°C and the residue dissolved in 2.5 ml of 0.1 M Tris-HCl (pH 7.4). Aliquots of 50-100 ul were taken for radioimmunoassay. Before the labled PGF_2 (New England Nuclear Corporation) was used it was purified on silicic acid columns (0.5g, 100 mesh).

Lung tissue content of PGF_2 was also measured. Seven rabbit lungs were perfused with horse plasma, 3 of which served as controls. Control lungs were perfused for 15-30 min without elevating outflow pressure, and frozen during a period of

decreasing or stable weight. In 4 lungs P_{LA} was raised to 15 or 20 mmHg and maintained at that pressure until the lungs were grossly oedematous.

Lungs were removed from the perfusion circuit and rapidly immersed in liquid DDM cooled to -155°C in liquid nitrogen in order to halt PG synthesis. Tissue pieces from the outer parts of the lower lobes were homogenized, (Dounce homogenizer, 2 min, 0°C) in 5 ml 0.15M NaCl and 0.1N HCl (5:2) to give a wet weight concentration of 200 ng/ml. 1ml of the homogenate was transferred to tubes containing 2.6 ml ethyl acetate:isopropanol (1:1) and mixed on a whirlmixer for 10 min. 3 ml 0.9% NaCl and 2 ml ethyl acetate were then added and the tubes were centrifuged (50,000g, 30 min, 4°C). 3 ml of the ethyl acetate phase was evaporated under nitrogen at 45°C, the residue dissolved in 2.5 ml 0.01M Tris-HCl (pH 7.4) and aliquots of 50-150 ul taken for RIA.

The concentration of PGF_2 in tracheal exudate was measured in duplicate samples from 7 rabbit lungs using the method outlined for plasma measurements.

SECTION 2. INDUCED INTRAVASCULAR PLATELET AGGREGATION IN THE INTACT CAT PREPARATION

METHODS

Cats weighing 3.0-5.1 kg were anaesthetized by i.p. injections of sodium pentobarbitone (30-40 mg/kg) and anaesthesia was maintained by further injections as required throughout the experiment. Animals were trachetomized and an i.v. injection of the muscle relaxant alcuronium chloride (alloferine 0.5 mg/kg) was given, and constant volume positive pressure ventilation was started (frequency = 24 strokes/min). End expiratory pressure (P_{EE}) was 1.5-2 cm H_2O and the tidal volume was adjusted to give an arterial pH of 7.40-7.45 at the start of each experiment. Tracheal pressure (equals transpulmonary pressure (P_{TP}) in the open chested animal) was recorded. Both femoral arteries and a femoral vein were cannulated for measurement of femoral arterial pressure (P_{FA}), for blood sampling and for administration of drugs respectively. Blood was obtained from donor cats (see p. 40) for infusion at the start of the extracorporeal circulation to compensate for blood loss.

Bioassay A detailed description of the extracorporeal circulation is given on p. 46 and will only be briefly outlined here.

Changes in arterial blood level of PGs were continuously monitored by the superfusion technique of Vane (1969). Cats were given 1000 I.U. heparin per kg, i.v. 30 min after the surgical procedures were finished. Ten ml/min of carotid arterial blood was pumped to superfuse the assay tissues rat stomach strip (RSS), rat colon (RC), chick rectum (CR),

a rabbit aorta strip (RbA) (five experiments) and a cat jejunum (CJ) in two experiments. Blood then drained by gravity back to the cat via a jugular vein. Prior to the experiment, tissues were bathed for 2-3 hr in Krebs Ringer solution containing 'combined antagonists' (p46). Isometric contractions of the tissues were recorded (p. 48). and calibrating doses of PGE_2, $PGF_{2\alpha}$, bradykinin and, in four experiments, angiotensin II were given as constant rate infusions for 4 min. In two experiments where the RbA was not included a strip of cat jejunum (CJ) was used to detect kinins.

Platelet Aggregation A suspension of collagen fibrils (1 mg/ml, 'Sigma') prepared as described by Holmsen, Day and Storm (1969) was infused i.v. at the rate of 0.2 ml/min for 6 min. If the initial collagen infusion caused only a small increase in peak inspiratory pressure (P_{PI}), the infusion rate was increased to 0.3 ml/min in each subsequent infusion to that animal.

Platelet Counting and Changes in the Number of Circulating Blood Platelets The number of circulating platelets was counted by the method of Brecher and Cronkite (1950). One ml of venous blood was collected and a fraction was diluted x 100 with 1% ammonium oxalate and examined under the light microscope in a counting chamber at magnification x 400. Ammonium oxalate haemolyzed the red blood cells and made the platelets more easily seen and identified. All platelets in 5 blocks of small squares were counted after they had settled for 15 min. Both sides of the chamber were counted and the number multiplied by 2,500 to give the number of platelets per cu. mm.

Experimental Protocol

When blood superfusion started, the cats were given a blood transfusion to compensate for the blood loss into the extracorporeal circulation. Throughout each experiment further transfusions of blood and Macrodex(Dextran T70 6% with NaCl 0.9%) were given whenever necessary to maintain a stable blood pressure. Several collagen infusions were given at 20-30 min intervals. Between infusions the assay tissues were calibrated with standard doses of PGs. Five to ten min before collagen infusions, the lungs were inflated for a few seconds to a peak inspiratory pressure of 20 cmH_2O by clamping the expiratory outlet tubing.

In 3 experiments the initial i.v. collagen infusion was followed by an infusion of collagen (0.05 ml/min) directly into the blood bathing the assay tissues.

In another 2 cats a series of collagen infusions were given until no or negligible airway responses could be elicited as described by Vaage, Bø and Hognestad (1974). At this stage blood superfusion of the assay tissues was started. Repeated collagen infusions (0.3 ml/min) were then given to the animals as described above.

Platelets

The number of circulating platelets was initially between 150,000 and 400,000 per mm^3 blood and always decreased during extracorporeal circulation. During the first hour it was reduced to 30-50% of the initial level, then gradually decreased in the next 1-2 hours to less than 50.000/mm^3 in most experiments. There was a further, temporary, fall in the number of circulating platelets during the first one or two collagen infusions. The fall in platelet count after later infusions was not significant since severe thrombocytopenia was then present. It was technically difficult to count platelets at this stage, since the few that were present were found mostly in aggregates.

SECTION 3. HYDROSTATIC PULMONARY OEDEMA AND CHANGES IN LUNG COMPLIANCE

METHODS

The isolated rabbit lungs were prepared as described on p.40 and perfusion was begun. The perfusate was always heparinized horse plasma, to which was added papaverine (7.5-10 mg in a perfusate volume of 200-250 ml) immediately after the start of perfusion to prevent active vasoconstriction and thereby any increase in vascular resistance. Control P_{PA} was set at 10-15 mmHg and if, during an experiment, P_{PA} was observed to increase steeply additional papaverine (5-7.5mg) was added to the reservoir.

Experimental Protocol and fixation:

Lungs were perfused at control pulmonary vascular and tracheal pressures. Peak inspiratory pressure (P_{PI}) was between 4.7 and 8.5 cmH_2O (P_{EE}=0.5-2.0 cmH_2O). The length of the control period depended on the pattern of weight change which was measured continuously as described (p.44). When the period of decreasing or stable weight had been obtained P_{LA} was raised by clamping the ladder of tubes at the venous outflow to 10, 15 or 20 mmHg weight increased by 9 to 18 g in the first 30 sec, indicating an increase in blood volume. P_{PI} increased simultaneously by about 25%. A change in P_{PI} during ventilation at constant tidal volume and normal respiratory frequency is a measure of the change in compliance of the lungs (C_L).

The initial steep decrease in C_L on raising P_{LA} (primary fall in C_L) is followed by a period of constant or slightly decreasing C_L

(i.e. constant or slightly increasing P_{PI}). After between 8-14 g of fluid has accumulated in the lungs (p. 38) the change in C_L begins to accelerate and this is called the secondary fall in C_L (Hauge, Bo and Waaler, 1975).

The pattern of weight increase was described on p.45. There was an initial steep rise for 30 sec to 2 min after the increase in P_{LA}, which then decelerated until a constant rate of weight increase was reached. This second rate of change is due to fluid filtration from the exchange vessels. The rate of increase depended on the height to which P_{LA} was raised. When rate was slow (less than 0.05 to 0.1g/min) P_{LA} was raised by a further 5 mmHg, causing a further similar pattern of weight gain, and a faster rate of filtration. When measuring changes in weight in these experiments, the initial increase was excluded and the difference between the final weight and the preparation weight 30 sec after increasing P_{LA} was used. In those experiments where a second increase in P_{LA} was performed, the contribution to weight gain of the increased blood volume during the first 30 sec was also subtracted. The 30 sec point was chosen as a reference and used in all experiments.

In experiments where filtration was very fast P_{LA} could be lowered by 5 mmHg, causing a steep initial decrease in weight then a steady rate of filtration which was slower than the previous one.

If filtration was allowed to continue it was usually constant but in some cases was seen to accelerate. This was also noted in lungs perfused for the measurement of PGs, and was seen to occur in all lungs where gross oedema was allowed to develop.

To arrest the situation at a chosen point of weight increase in order to quantify oedema in these preparations, the lungs were fixed

either by

a) perfusion with a solution of gluteraldehyde, or

b) rapid freezing in liquid arcton (dichlorodifluoro-methane, DDM).

a) Perfusion Fixation

The plasma perfusate to the lungs was exchanged with a 2.7% Krebs Ringer dextran (KRD) solution for a period of about 0.5-1.5 min until the perfusate was clear. The dextran solution usually had the same osmotic pressure as plasma and thus did not change the pattern of weight increase of the preparation. In some experiments the osmotic pressure of the dextran solution was higher than that of plasma and weight was stable or increased only slightly during this period. However, KRD perfusion time was kept as short as possible, and the weight increase during this time was included. Perfusions with a solution of 1.7% glutaraldehyde in Sörensens phosphate buffer was begun, and the first 50 ml of fixative leaving the lungs was discarded. Ventilation was stopped at end expiration and the perfusion continued for 15-20 min, during which period the lungs became stiff and yellow. When fixative perfusion was begun, the flow to the lungs was adjusted to keep the same P_{PA} as before.

Lungs were then removed from the perfusion circuit and tissue pieces about 1-2 cm^3 were cut from 2 (or 3) regions of the fixed lungs and dissected into 30 to 35 pieces 2x3x1.5-2 mm. Samples were taken from:

1) bottom lower lobe

2) upper lower lobe or middle lobe (not always taken)

3) upper lobe.

Tissue was immersed in 3% glutaraldehyde solution for one hour, placed

in Tyrode solution for 15 min then in 1% osmium tetroxide in Tyrode for 90 min in the refrigerator. Tissue was returned to Tyrode buffer for 15 min and fresh Tyrode for 15 min. It was then dehydrated by immersion in solutions of ethanol and propylene oxide:

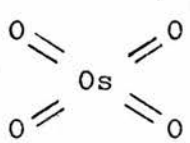
60% ethanol	60 min
96% ethanol	60 min
96% ethanol	60 min
100% ethanol	60 min
100% ethanol	60 min
propylene oxide	60 min
propylene oxide	60 min in filled air-tight plastic containers.

The tissue samples could then be stored in a 1:1 mixture of propylene oxide and epon at 3°C until further use.

Samples were embedded in epon by evacuating for up to 12-18 hours in a vacuum pump until all the air had been removed. In order to prevent hardening of the epon this was done in a cold room (3°C). The tissue pieces were transferred singly into fresh epon in individual plastic moulds, labled and allowed to harden by placing in an oven at 65 ± 3°C for 36-48 hours.

Osmium Tetroxide Osmium tetroxide is highly volatile, is a strong oxidizing agent and is reduced, by even small amounts of organic material and exposure to light, to the hydrated dioxide. As this is no longer a fixative, every care was taken to prevent this process occurring.

Osmium tetroxide (OsO_4 or osmic acid)



Osmium tetroxide acts as an electron stain. Reduced osmium stains

osmophilic structures such as proteins, which it stabilizes, and prevents them being coagulated by alcohols during dehydration. Osmium reacts with unsaturated fatty acids but not with DNA or RNA or most carbohydrates.

Osmium/Sörensens Phosphate Buffer Solution An osmium ampoule containing 0.1 g of oxmium tetroxide crystals was broken by a heavy glass rod inside a glass bottle containing 10 ml Tyrode solution. All glass surfaces were previously cleaned with nitric acid, rinsed thoroughly in distilled water and dried in a warm oven. A tight glass stopper was placed on the bottle, the solution was shaken vigorously and after half an hour the osmium tetroxide crystals dissolved. The bottle was protected from light by wrapping in aluminium foil, and the solution was always stored in the refrigerator. All procedures involving the use of osmium tetroxide were carried out in the fume cupboard.

Tissue samples were small (max 3 mm^2 x 0.5-1 mm) in order that osmium stain would be uniform. Osmium stain is even to a depth of 0.5-1 mm and larger blocks did not stain uniformly by immersion.

Embedding of glutaraldehyde-osmium fixed tissue. Epon 812 is a commonly used embedding medium (Kushida, 1959; Finch, 1960; and Luft, 1961). Epon 812 is a light coloured, glycerol based aliphatic epoxy resin of quite low viscosity (90-150 cps) and penetrates tissue quite quickly due to its low viscosity. It hardens uniformly at fairly low temperatures when mixed with acid anhydrides (dodencenyl succinic anhydride (DDSA) and nadic methyl anhydride (NMA) and an amine accelerator, 2, 4,6 tri- (dimethyl aminonethyl) phenol (DMP-30).

When the relative quantities of DDSA and NMA are varied the hardness of the plastic changes and a suitable hardness to match the specific

tissue type can be chosen.

The cutting quality of epon blocks is influenced by a combination of 3 factors: anhydride/epoxy ratio, final block hardness and the temperature and duration of polymerization. Cutting quality depends on the ratio of anhydride chemical equivalent to epoxy chemical equivalent in the mixture, therefore the exact content of epoxide in epon must be known.

Epon 812 was of standard epoxy equivalent 155 (i.e. weight of Epon in grams containing 1 gram chemical of epoxy). Luft (1961) found that easier sectioning results from a low anhydride/epoxy ratio in resin. The ratio should be 0.7-0.6 for best cutting quality (Coulter, 1967).

The amount of accelerator (DMP-30) is crucial for satisfactory polymerization (setting). Too much causes the block to become dark and brittle. Therefore this component was measured very carefully (see Hayat, 1970).

Epon was stored in batches of approximately 20 ml in the deep freeze at -20°C.

Preparation of Sections for Light Microscopy Epon blocks were trimmed until the tissue lay at the surface of the block and the four sides were shaped inwards at an angle of about 45° to this surface to prevent blunting of the knife.

Semithin sections approximately 1 μ thick were cut on a microtome using 45° angled glass knives with a water-filled waxed metal foil boat to float the sections. Sections were removed with a sharp stick and placed on a drop of water on glass microscope slides. Slides were heated on a hot plate at 55-60°C and allowed to dry at this temperature for 20-30 min.

Staining To obtain an overall staining of tissue components sections

were stained with toluidine blue (Trump, Smuckler and Benditt, 1961). A saturated solution of toludine blue in Sörensens phosphate buffer pH 7.2 (see Appendix p. 190) was made up. Several drops of this solution were placed on the slide (on hot plate at 60°) for 1-3 min before being rinsed with distilled water. "Depex" (1:1 mixture of depex and xylene solution was used to permanently mount cover slips.

b) Rapid Freezing Fixation :

Freezing. Lungs were perfused and the weight gain measured exactly as described for the perfusion-fixation experiments. At the required point of weight gain, the perfusate inflow and outflow of the lungs were clamped and ventilation was stopped simultaneously. The preparation was cut down and removed from the perfusion circuit and immersed as quickly as possible in liquid DDM cooled to about -150°C by a jacket of liquid nitrogen (-180°C). To ensure that the freezing process was as rapid as possible the DDM was stirred continuously.

The frozen lungs were then transferred to liquid nitrogen and pieces about 1 cm^3 were removed from the outer edges of the upper part of the lower lobes just below the hilum. Pieces were also taken from the lower part of the lower lobes. Samples were not taken from the upper lobes as these were often distorted in the clamping and freezing procedure. Freezing is fastest in the outer 2 mm of the lungs (Staub and Storey, 1962). However, light microscopic examination of the samples taken showed that structure in the parts examined was preserved in good condition for the observation of oedema (figs. 26 to 57).

Freeze Drying Two methods of freeze drying were employed.

1) Frozen tissue, approximately 1 cm^3, was placed on a grid surrounded by a heating element and suspended inside a "quick-fit" glass jar. The jar was closed (sealed with silicone high vacuum grease) and

immersed in liquid nitrogen. The jar was evacuated to a pressure of 0.001 mmHg and the grid was heated to -30 to -35°C. As ice sublimated from the tissue it condensed on the inside of the glass jar. The tissue dried in 24-30 hours. The heater was then switched off and, with the vacuum still functioning, the jar was allowed to heat to room temperature until the ice crystals inside sublimated. A liquid-nitrogen filled flask in series between the jar and the vacuum pump trapped the water. Tissue pieces could then be carefully removed by forceps into a dessicator and stored in airtight containers with silica. Macroscopic examination of dried tissue showed large bronchi and vessels. Cuffs of interstitial fluid around the vessels and bronchi could also be observed.

2) Tissue pieces were also dried in a freeze drier (Hetosicc, Hito Birkeröd, Denmark). Before drying tissue was stored for at least 24 hours at -70°C. The vacuum produced was approximately 0.1 torr. Pieces were dried for 18-24 hours, and many samples could be dried simultaneously.

Embedding of Freeze-Dried Tissue Freeze-dried tissues were embedded by two methods:

1) Epon embedding

2) Paraffin embedding.

1) Epon Embedding

Dried tissue was carefully cut into 2 mm cubes with a razor blade, taking care not to press or squeeze the tissue. Pieces were evacuated in epon for approximately 48 hours in a cold (3°C) room.

Cutting and Staining Tissue was embedded in epon blocks, as before, but without osmium stain. Sectioning of the tissues was the same although they were more difficult to section, possibly because they

were softer (osmium hardened the tissue). A modified epon mixture was used (see Appendix p.190) for the freeze-dried material. Blocks were sectioned and stained with toluidine blue as for glutaraldehyde-osmium fixed tissue.

2) Paraffin Embedding

Blocks of freeze-dried tissue (1 cm3) were placed directly into moulds containing melted (58-62°C) "Paraplast" paraffin wax, and kept in an oven at this temperature for 3 days, then cooled to room temperature. Sections 7-15 μ thick were cut on a microtome and placed on a solution of albumin-glycerine in water on glass microscope slides. This solution was allowed to evaporate to dryness on a warm plate (40°C) and the slides were then placed in an oven (37°C) overnight.

Removal of Paraffin and Staining Procedure

Slides were dipped in beakers containing the following solutions for the stated times to remove paraffin from the sections and to stain them for light microscopy (LM).

xylol	2-3 min
xylol	2-3 min
100% ethanol	2-3 min
100% ethanol	2-3 min
96% ethanol	2-3 min
70% ethanol	2-3 min
distilled water	2-3 min
hematoxylin	5 min
distilled water	10 min
eosin	1 min
distilled water	10 min

70% ethanol	2-3 min
96% ethanol	2-3 min
100% ethanol	2-3 min
100% ethanol	2-3 min
xylol	2-3 min
xylol	2-3 min

Coverslips were permanently mounted with dammer xylene solution.

SECTION 1. THE RELEASE OF PG's FROM LUNGS DURING INCREASED HYDROSTATIC PRESSURE AND OEDEMA

RESULTS

Bioassay of Perfusate from Isolated Lungs

A total of 22 rabbits, 6 cats and 3 guinea-pigs were used. The results of the bioassay of prostaglandins in isolated lungs are shown in Tables 1 and 2. The lungs were divided into two groups according to the method used to induce pulmonary oedema.

Group 1

Group 1 contains those lungs where oedema was induced by raising vascular hydrostatic pressure. Table 1 shows the results from this group (19 experiments). The species of lung donor, type of perfusate, values to which P_{LA} was raised and the total perfusion time (or time to release of PG if this occurred) is shown. The inclusion of the RbA and the addition of indomethacin to the lung perfusate are also tabulated. Depending on the values of P_{LA}, group 1 is divided into 2 subgroups (A and B): slow and rapid induction of oedema.

A) Gradual Development of Oedema

In 10 cat and rabbit lungs perfused with horse plasma (8 lungs) or Krebs Ringer Dextran solution (KRD) (2 lungs) P_{LA} was initially raised to 10 (n=6) or 15 (n=4) mmHg for between 5 and 20 min to induce a slow filtration of fluid from the pulmonary exchange vessels into the interstitium.

In 7 plasma-perfused lungs (No. 1-5 and 7 and 8, Table 1) P_{LA} was raised and maintained or further increased to a maximum of 25 mm Hg until gross alveolar oedema was observed. At this stage more than

TABLE 1.

Duration of increased P_{LA} and release of prostaglandins in isolated lungs.
(*= RbA included in cascade).

Group Exp. no.		Species	Perfusate	Duration of P_{LA} elevation (min) 10 mmHg	15 mmHg	20 mmHg	25 mmHg	30 mmHg	Total perfusion time(min)	Release of PG Time (min)	Assay as PGE_2 (ng/ml)	Indometh into reservoir
1A	1	rabbit	horse plasma	10	10	10			184			
	2	rabbit	horse plasma	10	18	41			187			
	3	rabbit	horse plasma		21				173			
	4	rabbit	horse plasma		10		8		147			
	5	rabbit	horse plasma		13	15	10		174			
	*6	rabbit	horse plasma	10	23+59	39			180			25µg/ml
	7	cat	horse plasma	7		12			175			
	8	cat	cat plasma	5	7	38			249			
	9	rabbit	KRD	12	31					60	1.0	10µg/ml
	10	rabbit	KRD		10	15				80	0.5-1.0	
1B	11	cat	cat plasma			33	15		191			
	12	cat	horse plasma			8	14		133			
	*13	cat	cat blood				15		301			
	*14	cat	cat blood				50		163			
	15	guinea-pig	horse plasma				10	5	105			
	16	guinea-pig	horse plasma				10		175			
	17	guinea-pig	Krebs Ringer				12		104			
	*18	rabbit	horse plasma				3		105			125 µg/m
	19	rabbit	KRD			20				80	0.5	

50g of fluid had accumulated extravascularly. In no experiment could any contraction of the assay tissues be observed during or after oedema development.

In one pair of lungs perfused with horse plasma (No.6, Table 1) P_{LA} was first raised to 10mmHg for 10 min then to 15mmHg for 23 min and returned to control, normalizing blood volume and starting fluid reabsorbtion. Repeated pressure elevations were made (Table 1).

A total of 4 pressure elevations were performed in these lungs. The third and fourth are shown in figs. 6 and 7 respectively, where P_{LA} was 15 and 20 mmHg. The end result was fulminant alveolar oedema with fluid pouring out of the trachea to such an extent that ventilation had to be stopped. At this point the perfusate was gassed with 5% CO_2 in air. No contractions of the assay tissues were observed during the period of increased P_{LA}.

In this experiment tissue superfusion with the venous effluent was temporarily interrupted and immediately replaced by the tracheal fluid. This caused only a short-lasting contraction of the RSS (fig. 7), and a similar contraction, which was also not maintained, occurred when the venous effluent replaced the tracheal fluid as a superfusate. These contractions may have been due to changes in gas tension or pH in the superfusate. Basal tissue tension was the same in tracheal fluid and plasma. When indomethacin was added to the reservoir (10µg/ml) it did not relax the assay tissues, showing that no PG synthesis had occurred.

Two lungs were perfused with KRD solution (Table 1, No. 9 and 10) and P_{LA} was increased. Lung No 9 was perfused for 60 min during which time weight was stable. P_{LA} was raised to 10mmHg for 12 min

Fig. 6. Rabbit lungs perfused with horse plasma. The third of four periods of left atrial pressure elevation is shown. Left atrial pressure (P_{LA}) was raised to 15mmHg and maintained at that value. The tracings show the isometric tensions of the bioassay tissues: rabbit aorta (RbA), rat stomach strip (RSS), rat colon (RC) and chick rectum (CR). Pulmonary arterial pressure (P_{PA}) and the changes in weight (in grams) of the preparation are shown (Δ Wg). The sensitivity of the assay tissues was tested by infusing PGE_2 and $PGF_{2\alpha}$ (DIR) into the superfusate before the period of pressure elevation.

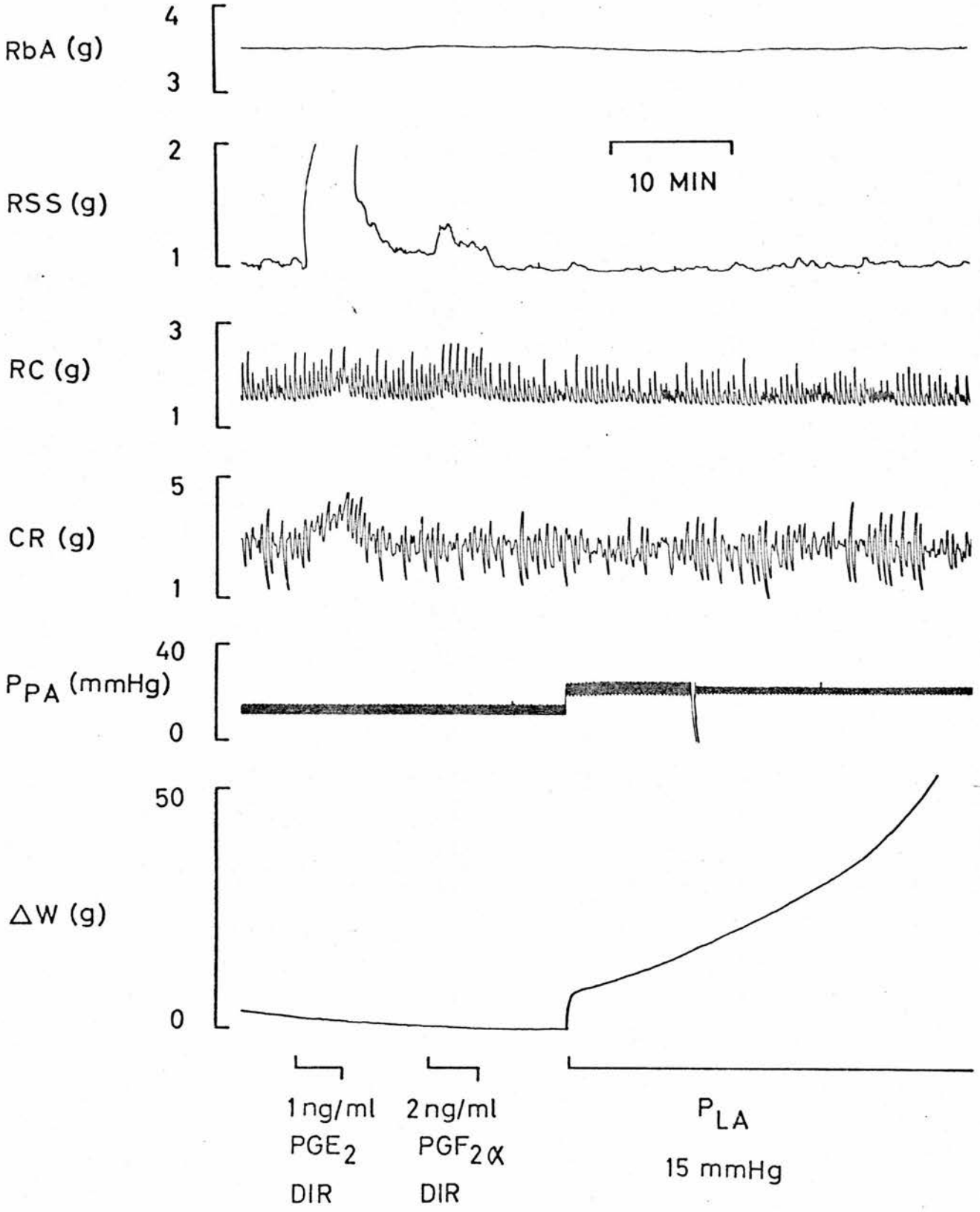

RbA (g)
4
3
RSS (g)
2
1
10 MIN
RC (g)
3
1
CR (g)
5
1
P_{PA} (mmHg)
40
0
ΔW (g)
50
0
1 ng/ml
PGE_2
DIR
2 ng/ml
$PGF_{2\alpha}$
DIR
P_{LA}
15 mmHg

Fig. 6.

Fig. 7. Shows the tensions (in grams) of the assay tissues during the fourth (and final) period of pressure elevation in the experiment also depicted in Fig.6. RbA= rabbit aorta, RSS= rat stomach strip, RC= rat colon and CR= chick rectum. Pulmonary arterial pressure (P_{PA}) is shown. The tissues were calibrated with direct infusions of PGE_2 and $PGF_{2\alpha}$. Left atrial pressure was raised and maintained at 20mmHg (P_{LA} 20mmHg). Tissue superfusion with venous effluent was temporarily stopped and replaced with tracheal fluid for a period of 8 min. Indomethacin (10mg IR) was added to the reservoir at the end of the experiment. Due to an electrical disconnection on the pen recorder, the tracing of the RC was temporarily interrupted.

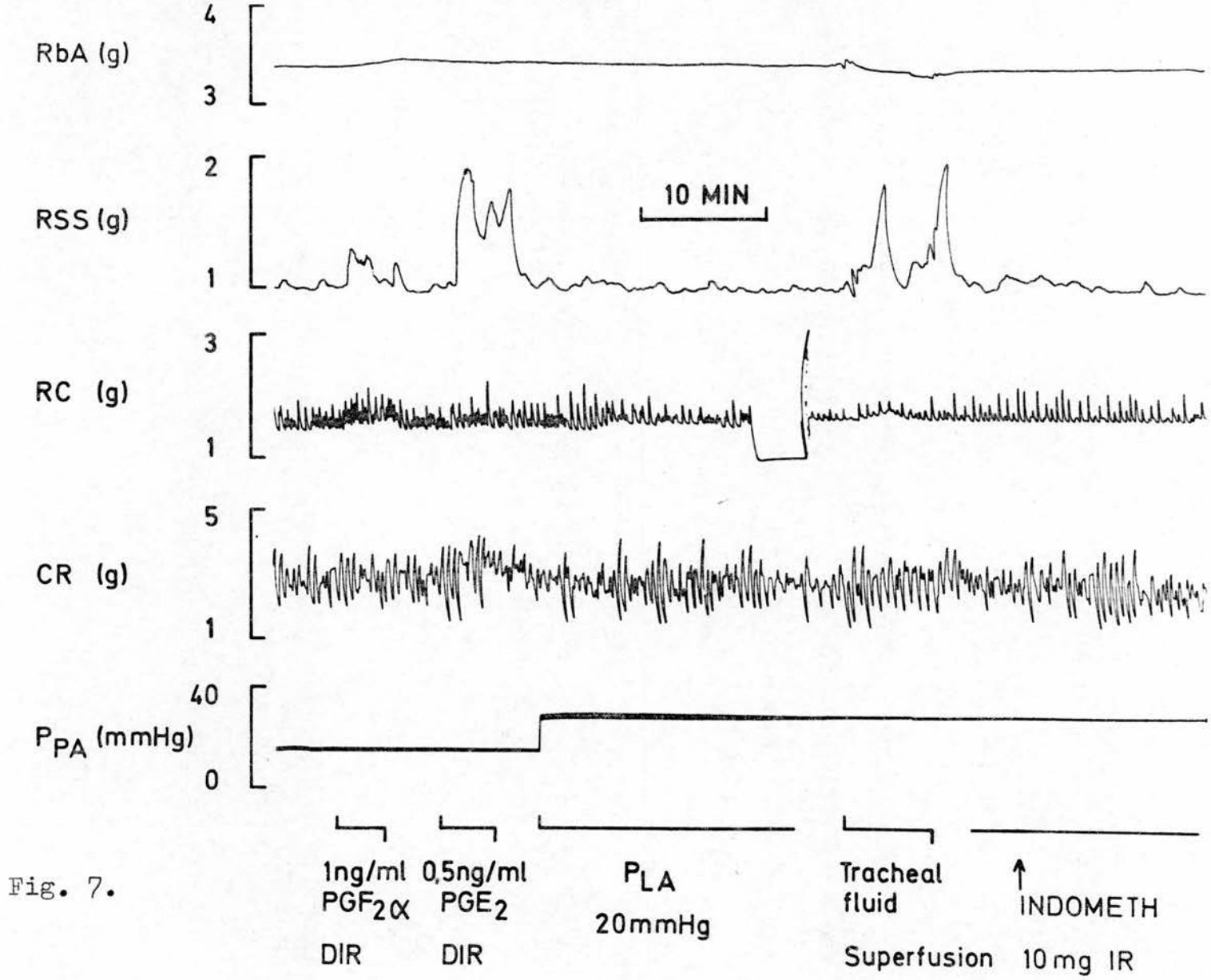

RbA (g)
4
3
RSS (g)
2
1
10 MIN
RC (g)
3
1
CR (g)
5
1
P_{PA} (mmHg)
40
0
1ng/ml $PGF_{2\alpha}$
DIR
0,5ng/ml PGE_2
DIR
P_{LA}
20mmHg
Tracheal fluid
Superfusion
INDOMETH
10 mg IR

Fig. 7.

then raised to 15 mmHg and maintained at that pressure. Approximately 75 min after the start of perfusion a progressive release of PGLS began. Baselines and activity of RSS and CR increased. This was assayed as 1ng/ml PGE_2. Indomethacin (10µg/ml) was added to the lung perfusate and the assay tissue tensions returned to baseline, confirming that the tissue activity had been due to PGLS.

Lung No. 10 was perfused for 72 min before P_{LA} was raised (Table 1). PG release was detected (during P_{LA} elevation) 80 min after the start of perfusion and increased to a value of 0.5-1ng/ml PGE_2.

B) Rapid Development of Oedema

In a total of 9 cat, rabbit and guinea-pig lungs perfused with blood, horse plasma or Krebs Ringer dextran solution, P_{LA} was initially raised to 20 (n=3) or 25 (n=6) mmHg. In one experiment P_{LA} was increased further to 30 mmHg (Table 1). These manoeuvres caused marked pulmonary hypertension and rapid onset of gross alveolar oedema. However, in the blood, plasma or KR perfused lungs contractions of the assay tissues during vascular hypertension and lung oedema were never observed, indicating that no release of PGLS had taken place.

Fig. 8 shows the results from one experiment (lung 18) where P_{LA} was increased to 20 mmHg for 3 min and then returned to zero. Weight rose rapidly during the period of raised P_{LA}. A net weight gain of 50 g remained after the vascular pressure was normalized, representing irreversible extravascular fluid accumulation. The assay tissues (RbA, RSS, RC and CR) were calibrated before the after the period of pressure elevation with doses of 0.5 and 1 ng/ml PGE_2 and $PGF_{2\alpha}$. There was no PG release detectable on the tissues due to vascular distension or to pulmonary oedema. Indomethacin (50 mg) added to the

Fig. 8. A pair of rabbit lungs perfused with horse plasma. The tracings show tracheal pressure (P_{TP}), pulmonary arterial pressure (P_{PA}) and the changes in weight of the preparation (ΔW). Isometric tensions (in grams) of the following bioassay tissues, superfused with venous effluent, are also shown: rabbit aorta (RbA), rat stomach strip (RSS), rat colon (RC) and chick rectum (CR). The scale of the RC recording was changed during the experiment. Calibrating doses of $PGF_{2\alpha}$ and PGE_2 were infused into the superfusate upstream to the assay tissues (DIR). P_{LA} was increased to 20 mmHg for 3 min then returned to control. Indomethacin (10, 20 and 20 mg), added to the reservoir, did not relax the assay tissues.

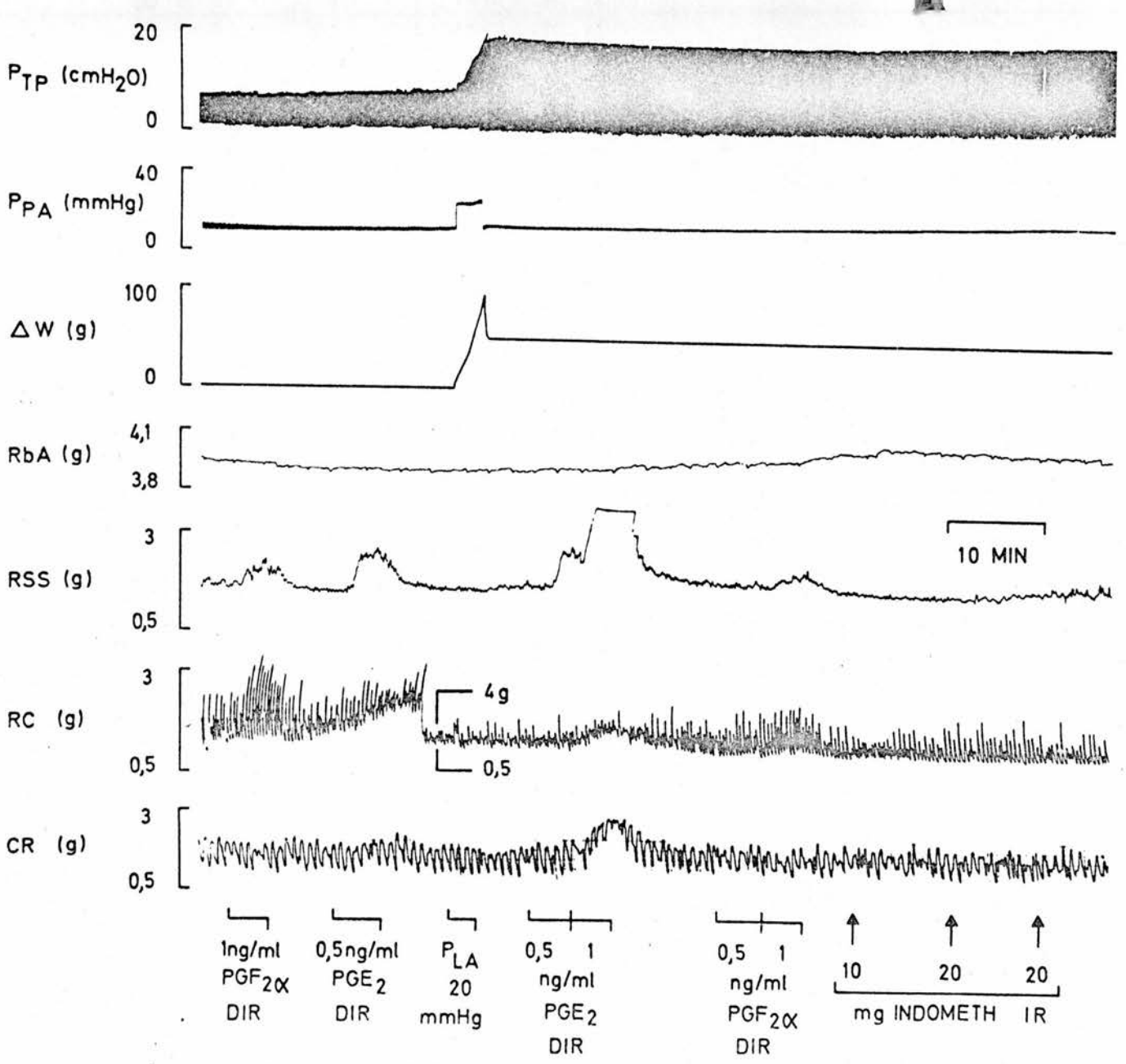
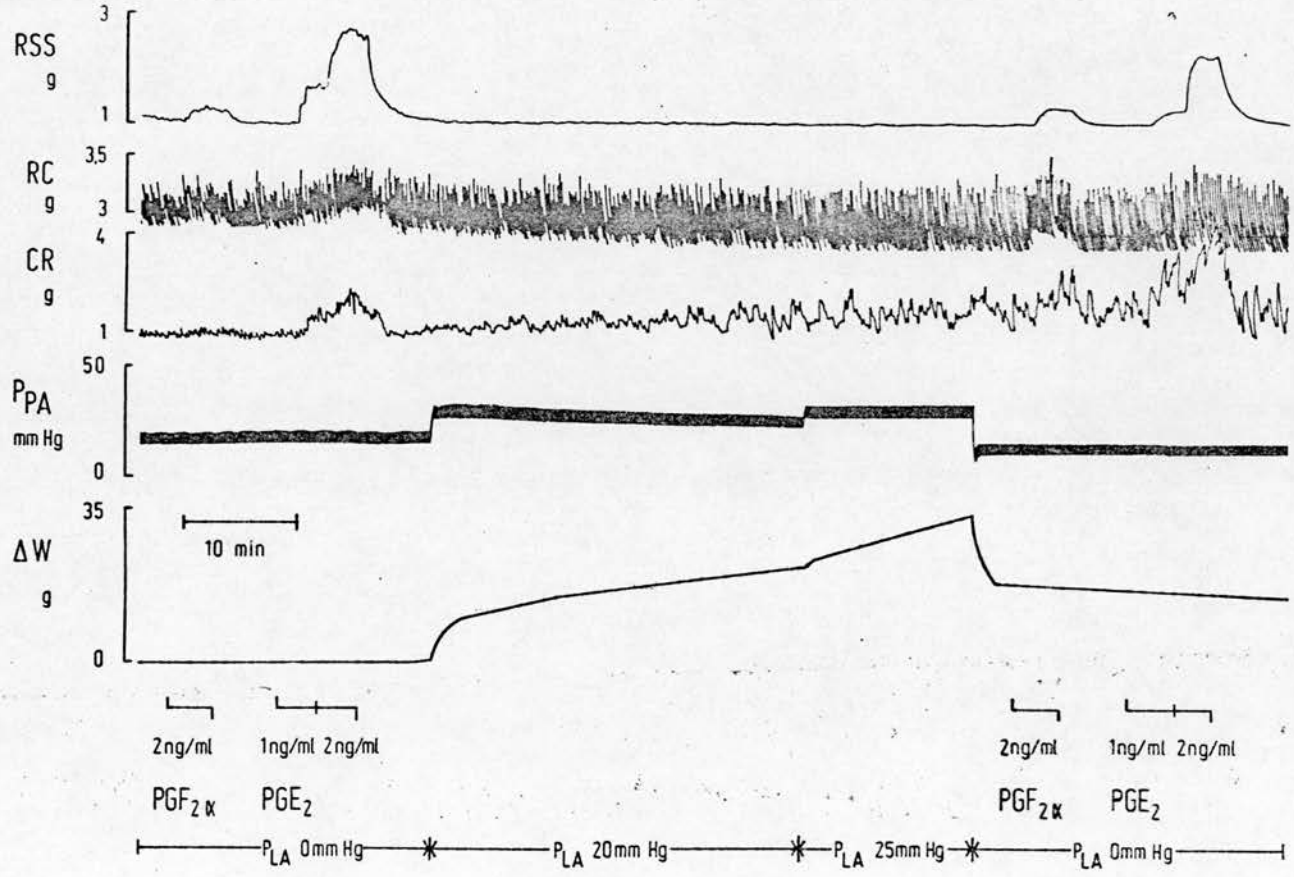

20
P_{TP} (cmH_2O)
0
40
P_{PA} (mmHg)
0
100
ΔW (g)
0
4,1
RbA (g)
3,8
3
RSS (g)
0,5
10 MIN
3
RC (g)
0,5
4g
0,5
3
CR (g)
0,5
1ng/ml PGF2α DIR
0,5ng/ml PGE2 DIR
PLA 20 mmHg
0,5 1 ng/ml PGE2 DIR
0,5 1 ng/ml PGF2α DIR
10
20
20
mg INDOMETH IR

Fig. 8.

Fig. 9. Shows an isolated cat lung perfused with cat plasma. The tension of the assay tissues, superfused with 10ml/min of the venous effluent from the lungs, is shown. RSS= rat stomach strip, RC= rat colon, CR= chick rectum. Pulmonary arterial pressure (P_{PA}) and the change in weight of the preparation (ΔW) are shown. Assay tissues were calibrated with doses of PGE_2 and $PGF_{2\alpha}$ given directly into the superfusate. Left atrial pressure (P_{LA}) was raised to 20 and 25 mmHg for 33 and 15 min respectively, and returned to control.



reservoir, did not relax the assay tissues. Fig. 9 shows the results from a cat lung perfused with cat plasma (Table 1, No. 11) where P_{LA} was raised to 20 (33 min) and to 25 mmHg (15 min). No release of PGLS was detectable.

In experiment No. 19, rabbit lungs were perfused with KRD for 58 min. P_{LA} was raised to 20 mmHg for a period of 20 min and returned to control. Perfusion then continued with control vascular pressures for approximately 3 min before release of PGLS was detected. Release was observed as a progressive increase in the baselines of the assay tissues RSS and RC, and was assayed as up to 0.5ng/ml PGE_2.

Group 2

The method of inducing oedema formation in this group of 12 rabbit lungs was to perfuse the lungs with KRD solution, KR, plasma or KRD containing histamine blockers and monitor the changes in weight. During extended perfusion in isolated lungs the weight of the preparation begins to increase slowly then progressively faster and eventually alveolar oedema develops with fluid pouring from the trachea. The results of these experiments are subdivided according to the type of perfusate used:

(A) Perfusion with KRD Solution

Three pairs of lungs were perfused and hyperinflated to a P_{TP} of 20 cm H_2O for 3-5 sec at intervals of 10 to 20 minutes throughout the course of perfusion. Lungs were perfused for 30 to 55 min before weight increase began. After perfusion for 100, 100 and 150 min respectively, a spontaneous release of PGE_2-like substances (0.5-1 ng/ml) occurred. Moderate alveolar oedema was present in two of the lungs, but not in lung No. 1 (Table 2) where weight increase was only

Group and Experiment No.	Perfusate	Amount of PG released assayed as PGE_2 ng/ml	Time (min)		weight increase (g)		X Indomethacin µg/ml	
			To release	Total, in no release	At release	Total if no release		
2 A: 1	KRD	0.5-1.0	100		3.6			-
2	KRD	0.5	100		19.4			-
3	KRD	1.0	150		10.7		12.5	R
B: 4	Krebs Ringer	0		165	*	> 70		-
5	Krebs Ringer	0		130	*	> 50		-
6	Horse plasma	0		214	*	> 50	25	0
C: 7	KRD							
	H_1+H_2 blockers	0		213		> 50	125	0
8	KRD							
	H_1+H_2 blockers	0		260		> 60		-
D: 9	KRD							
	H_1 blocker	0		180		> 50		-
10	KRD							
	H_1 blocker	0		184		> 76	62.5	0
E: 11	KRD							
	H_2 blocker	0.5	188		30.8		12.5	R
12	KRD							
	H_2 blocker	0.5 o	129		5.0		25	R

* Experiments with RbA included
o Assayed also as 1 ng $PGF_{2\alpha}$ in this experiment
X Indomethacin into reservoir: effect on tissues
R = Reduction in tension to baseline
0 = No effect
- = None given

Table 2. Release of prostaglandins in isolated, perfused lungs.

3.6g. The results from one lung (No. 3) are shown in fig. 10. The assay tissues RSS, RC and CR were sensitive to calibrating doses of 0.5-1 ng/ml PGE_2 and 1-2 ng/ml $PGF_{2\alpha}$. Tissue baselines were stable at the start of perfusion (left hand column) and at this stage hyperinflations did not release PG-like substances (PGLS). Ninety min. later there was release of PGLS (right hand column), and stimuli such as hyperinflation, raised left atrial pressure (P_{LA}=15 mmHg for 10 to 20 sec) or increased perfusion flow (10 sec) caused additional release of PGLS corresponding to 0.5 ng/ml PGE_2. There was also a gradual increase in baseline of the tissues RSS and CR, which was reversed by the addition of 12.5 µg/ml indomethacin to the lung perfusate.

(B) Perfusion with KR Solution or Horse Plasma

Two preparations were perfused with KR solution and one with horse plasma for between 2-3.5 hr. Weight in the KR perfused lungs began to increase 15 to 20 min after the start of perfusion and the lungs rapidly became grossly oedematous. Eventually perfusate poured from the trachea and ventilation was stopped. No PG release could be detected throughout perfusion, and periods of hyperinflation (at intervals of approximately 20 min) did not stimulate PG synthesis (fig. 11). All lungs were oedematous by the end of the perfusion period (Table 2). That is, weight gains were large (greater than 50g).

(C) Perfusion with KRD Solution Containing Histamine H_1 and H_2 Receptor Blockers

In 2 pairs of lungs with mepyramine maleate (2.5 ng/ml) and metiamide (75 ng/ml) in the perfusate, no release of PGLS was detected during perfusion for 3.5 to 4.5 hr. Nor was there any release due to hyperinflation.

Fig. 10. Rabbit lungs (Table 2, exp. 3) perfused for 3hr with KRD solution and hyperinflated(H) to a maximum P_{TP} of 20 cm H_2O for 3-5 sec at intervals of 10 to 20 min. Pulmonary arterial pressure (P_{PA}) and the isometric tension (in grams) of the assay tissues: rat stomach strip (RSS), rat colon (RC) and chick rectum (CR) are shown. Calibrating doses of 1ng/ml PGE_2 and $PGF_{2\alpha}$ were given at the start of the experiment and after 90 min of perfusion. Left atrial pressure was raised to 15 mmHg for 10 sec (P_{LA}). Increased flow of perfusate to the lungs (Flow) was caused by raising pump speed for 10 sec until P_{PA} was similar to that during hyperinflation. Indomethacin (5mg) was added to the reservoir (IR).

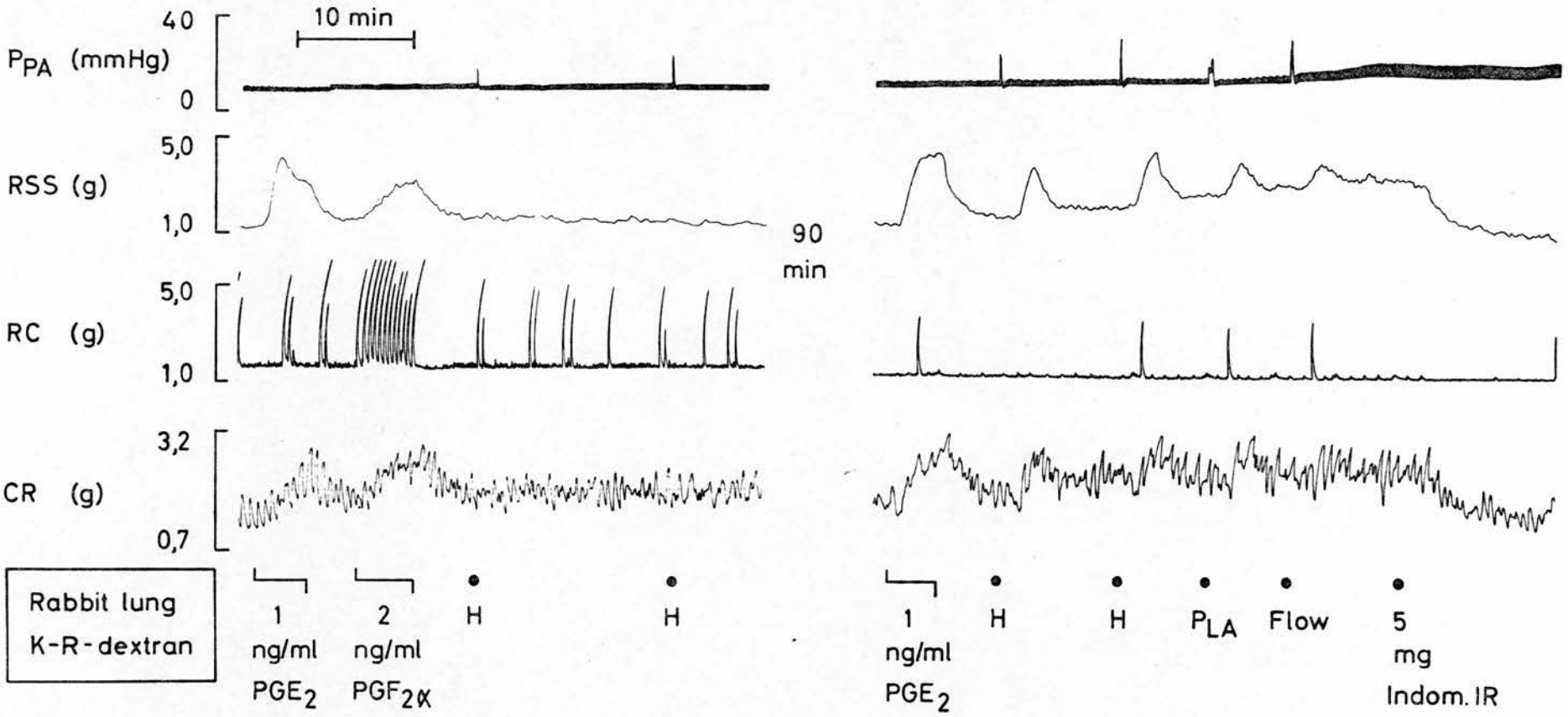

40
10 min
P_{PA} (mmHg)
0
5,0
RSS (g)
1,0
90
min
5,0
RC (g)
1,0
3,2
CR (g)
0,7
Rabbit lung
K-R-dextran
1
ng/ml
PGE_2
2
ng/ml
$PGF_{2\alpha}$
H
H
1
ng/ml
PGE_2
H
H
P_{LA}
Flow
5
mg
Indom. IR

Fig. 10.

Fig. 11. Rabbit lungs perfused with horse plasma. (Table 2, exp. 6). Tracheal pressure (P_{TP}) and pulmonary arterial pressure (P_{PA}) are shown. The assay tissues are rabbit aorta (RbA), rat stomach strip (RSS), rat colon (RC) and chick rectum (CR). Tissues were calibrated with 0.5-1 ng/ml PGE_2 and 2 ng/ml $PGF_{2\alpha}$. Periods of hyperinflation (H) throughout perfusion did not release PGs either at the start of perfusion (left side of fig.) or after 90 min (right side of fig.).

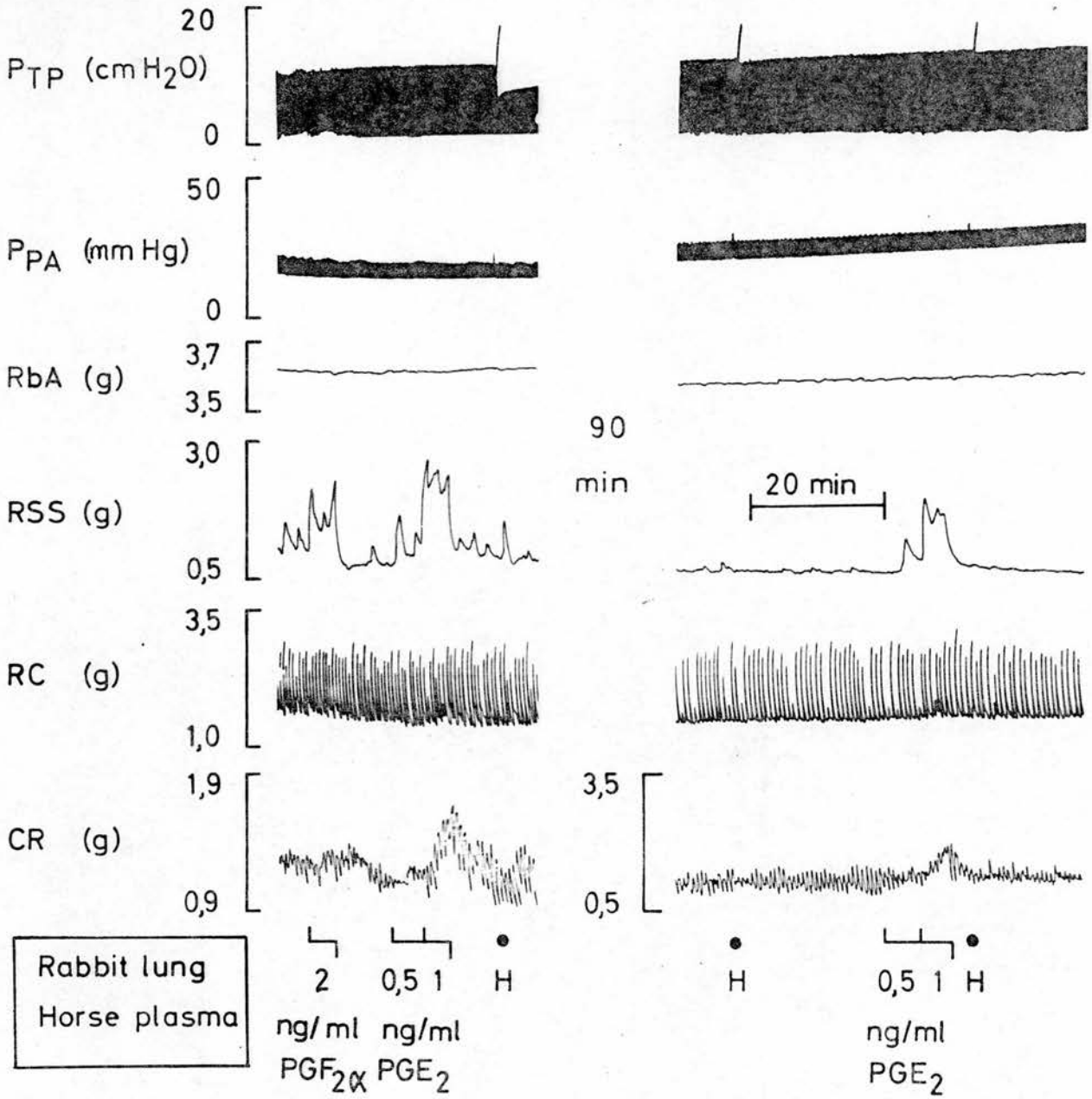

20
P_{TP} (cm H_2O)
0
50
P_{PA} (mm Hg)
0
RbA (g)
3,7
3,5
90
min
20 min
3,0
RSS (g)
0,5
3,5
RC (g)
1,0
1,9
CR (g)
0,9
3,5
0,5
Rabbit lung
Horse plasma
2
0,5 1
H
ng/ml
$PGF_{2\alpha}$
ng/ml
PGE_2
H
0,5 1 H
ng/ml
PGE_2

Fig. 11.

(D) Perfusion with KRD Solution Containing Histamine H_1 Receptor Blocker

Two pairs of lungs where mepyramine maleate (2.5 ng/ml) was added to the perfusate did not release PGs during perfusion for 3 hrs. Stimuli such as hyperinflations, short periods of increased P_{LA} or increased P_{PA} by increasing flow caused no release of PGLS. Fig. 12 shows the results from one such experiment where lungs had already been perfused for 90 min.

(E) Perfusion with KRD Solution Containing Histamine H_2 -Receptor Blocker

Two pairs of lungs perfused with KRD during H_2 receptor blockade released PGLS (assayed as less then 0.5 ng/ml PGE_2) beginning 2 and $2\frac{1}{2}$ hr after the start of perfusion. At this stage hyperinflations and elevations of vascular pressure caused additional PG-release (about 0.5 ng/ml of PGE_2). One such experiment is shown in fig. 13. Indomethacin added to the reservoir returned the tensions of the assay tissues to baseline and a subsequent period of increased P_{LA} had no effect on the tissues, confirming that PGLS had caused the tissue activity.

Bioassay of Arterial Blood in Intact Cats

Pulmonary and Cardiovascular Effects of Raised P_{LA}

When the balloon in the left atrium was inflated P_{LA} increased together with P_{PA} and remained elevated until the balloon was deflated. The pressure gradient across the lungs decreased markedly and pulmonary vascular resistance also decreased although cardiac output was reduced. Concomitantly P_{TP} increased and P_{FA} decreased.

Fig. 12. Rabbit lungs perfused with KRD solution containing 2.5 ng/ml mepyramine, a histamine (H_1) receptor blocker. Pulmonary arterial pressure (P_{PA}) and the tension (in grams) of the assay tissues RSS and CR are shown. This fig shows the situation after 90 min of perfusion. Tissues are calibrated by direct infusion of 1 ng/ml PGE_2 and 2 ng/ml $PGF_{2\alpha}$. Hyperinflation (H), a period of increased flow (Flow) and increased outflow pressure (P_{LA}) fail to release PGLS detectable on the assay tissues.

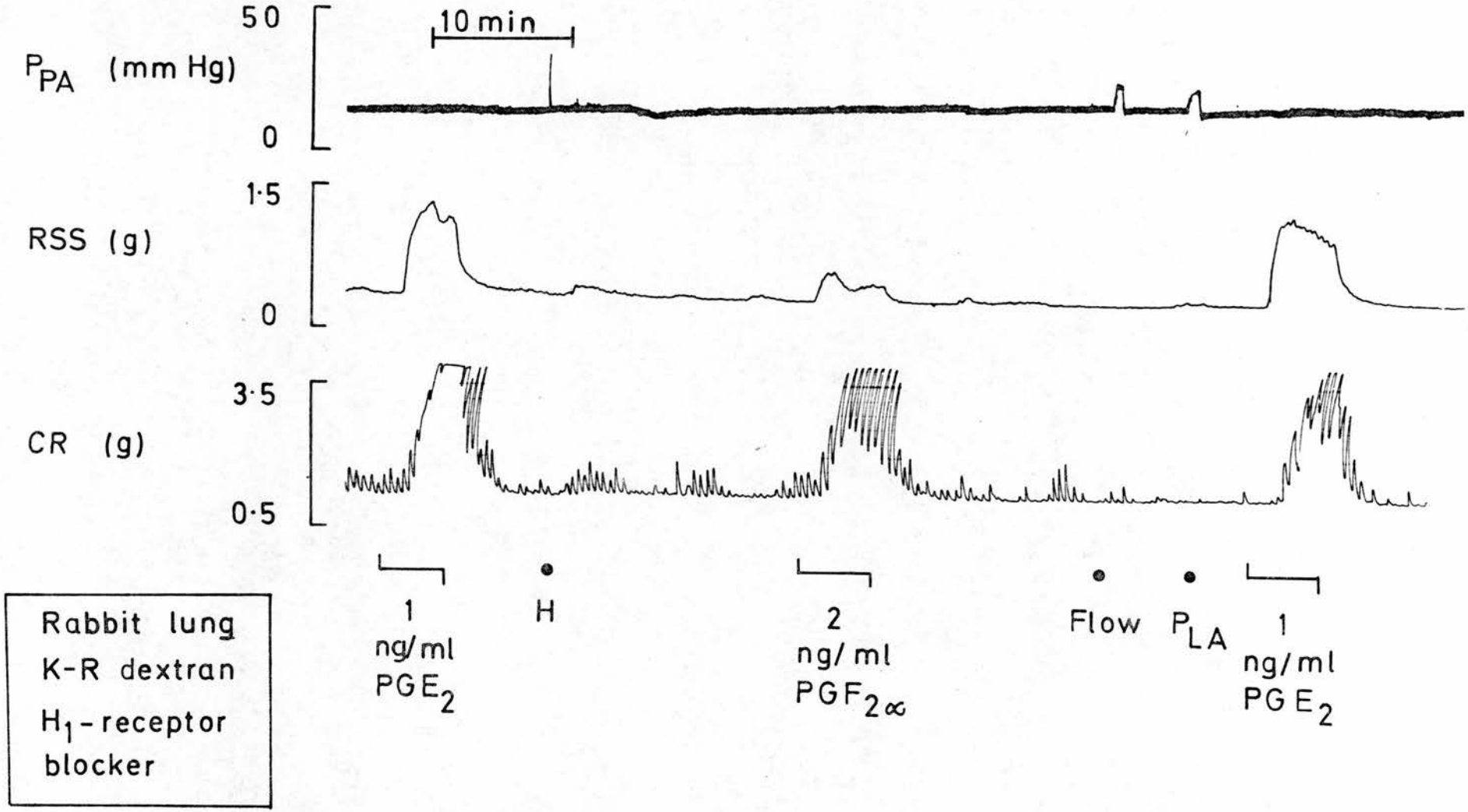

50
10 min
P_{PA} (mm Hg)
0
1·5
RSS (g)
0
3·5
CR (g)
0·5
1
ng/ml
PGE_2
H
2
ng/ml
$PGF_{2\alpha}$
Flow
P_{LA}
1
ng/ml
PGE_2
Rabbit lung
K-R dextran
H_1-receptor
blocker

Fig. 12.

Fig. 13. Rabbit lungs perfused with KRD solution containing 75 ng/ml of the histamine (H_2)receptor blocker, metiamide. The fig. starts after 90 min of perfusion. Perfusion pressure (P_{PA}), tracheal pressure (P_{TP}) and the isometric tensions of the assay tissues: rat stomach strip (RSS), rat colon (RC), and chick rectum (CR) are shown. The tissues were calibrated with PGE_2 and $PGF_{2\alpha}$. Hyperinflations (H) were performed at intervals of 10-20 min. Increases in outflow pressure (P_{LA}) and perfusate flow (Flow) released small amounts of PGLS before the addition of indomethacin (5 mg IR) into the reservoir.

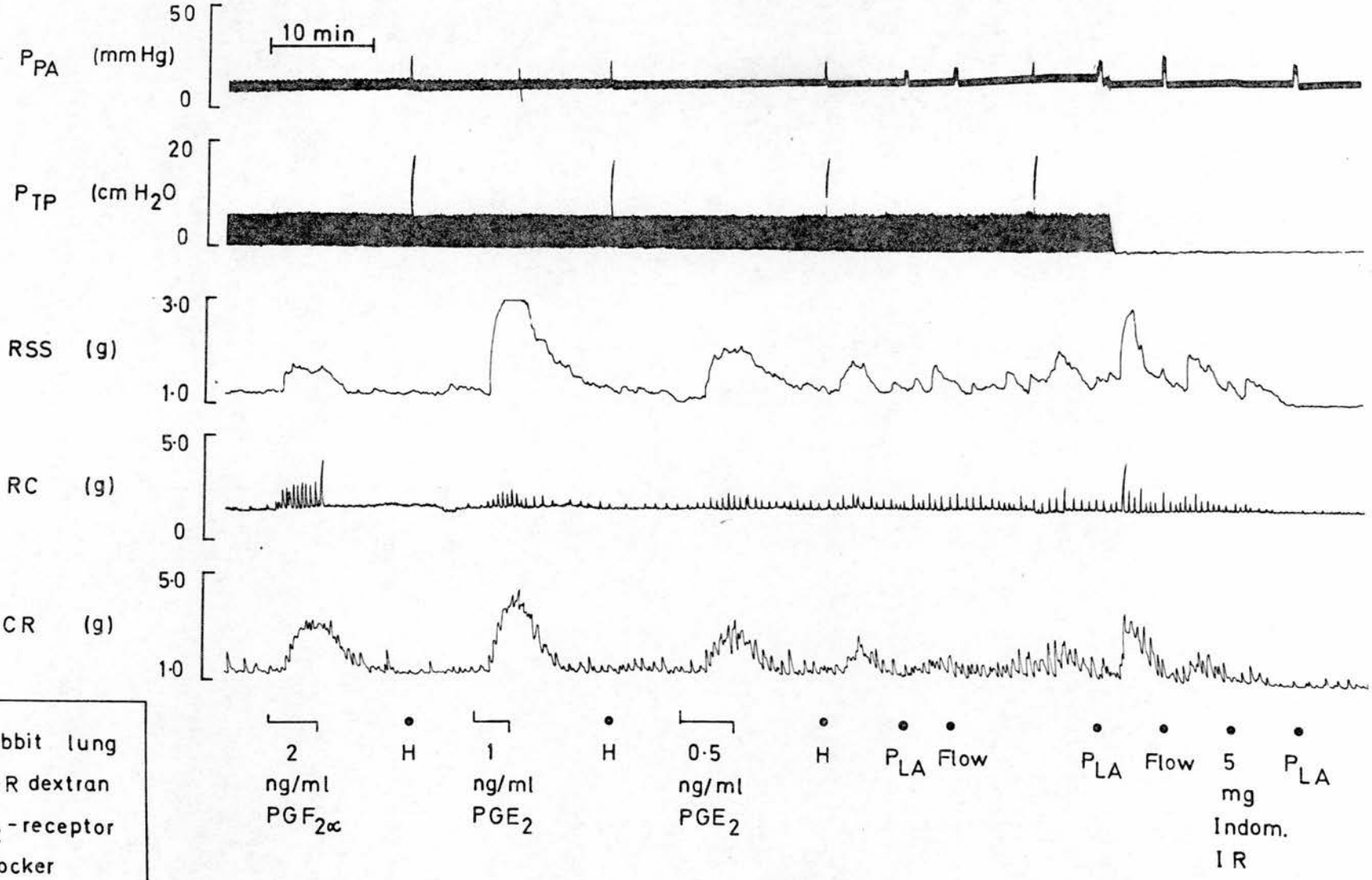

50
10 min
P_{PA} (mm Hg)
0
20
P_{TP} (cm H_2O
0
3·0
RSS (g)
1·0
5·0
RC (g)
0
5·0
CR (g)
1·0
2 ng/ml $PGF_{2\alpha}$
H
1 ng/ml PGE_2
H
0·5 ng/ml PGE_2
H
P_{LA}
Flow
P_{LA}
Flow
5 mg Indom. I R
P_{LA}
Rabbit lung
K-R dextran
H_2-receptor blocker

Fig. 13

Release of PGLS

A total of 18 pressure elevations were performed in 9 cats. The pressure elevations were in the range of 21-49 mmHg and lasted for 2-26 min (Table 3). Changes in P_{LA} and P_{FA} are also shown in the table. In 14 of the 18 pressure elevations contraction of assay tissues occurred indicating appearance of PG-like substances (PGLS) in arterial blood (fig. 14). In 4 of these 14 elevations the type of PG could not be specified since it did not exactly match a calibrating dose. In 10 elevations the assay tissues indicated release of $PGF_{2\alpha}$-like substances. Estimated as $PGF_{2\alpha}$, the concentrations ranged from 1 ng/ml to 4 ng/ml (mean 2 ng/ml). The tissues responded 2-3 min after P_{LA} was elevated and remained contracted in all but one case throughout the period of pressure elevation. When P_{LA} was reduced after a period of high pressure the tissues returned to their pre-elevation tension level. Tissue baselines were stable during the control periods between pressure elevations (usually about 30 min) with P_{LA} of 2-5 mmHg.

In 4 out of 18 pressure elevations release of PGLS could not be detected. These 4 elevations of P_{LA} were in 3 cats and in each of these cats release of PGLS could be measured during another P_{LA} elevation (Table 3). When no PGLS was detected the magnitude and duration of the pressure elevations were not different from elevations where release of PGLS occurred.

During 2 pressure elevations gross alveolar oedema developed, judged from foam pouring out of the trachea, and P_{TP} increased throughout pressure elevation and did not normalize afterwards. One of these cases was accompanied by release of PGLS whereas the other was not.

Table 3. Effects of increased P_{LA} in intact cats.

Cat no.	Pressure elevation no.	P_{LA} (mmHg)	Duration of P_{LA} elevation (min)	PG-like substances (estimated as PGF_2)	P_{FA} (mmHg) before & during P_{LA} incr.	Indo inf.
1	1	24	20	0	130-120	
1	2	42	6	0	130-120	
1	3	43	21	4	110-100	
1	4	25	2	1	80-70	
1	5	21	7	PGLS	70-65	
2	1	29	26	1	110-85	
2	2	26	21	0	110-85	
3	1	32	11	4	90-60	
4	1	30	10	2	140-85	
4	2	28	20	3	135-75	+
5	1	33	11	2	125-80	
5	2	30	24	2	95-75	+
6	1	30	16	PGLS	115-80	
7	1	49	15	2	115-80	
7	2	40	13	0	90-65	
7	3	30	10	PGLS	85-70	
8	1	30	12	2	90-70	+
9	1	31	21	PGLS	90-70	+

PGLS indicates that the type of PG could not be specified

Effect of Indomethacin

During 4 episodes of elevated P_{LA} with a definite increase in PGLS, indomethacin (2 mg/kg) was injected slowly i.v. to inhibit synthesis of PGs. Within 4-5 min the assay tissues relaxed to the original basal tensions and their tensions did not change when P_{LA} was reduced (fig. 14). Immediately after indomethacin was injected P_{FA} fell about 10% and remained at this reduced level.

In cats No 6 to 9 a RbA was included in the superfusion circuit. in the 6 pressure elevations in these cats the RbA contracted in 4 cases, whereas RSS, RC and CR indicated release of PGLS during 5 of the pressure elevations.

Release of Angiotensin

In all the cats a reduction in P_{FA} occurred when P_{LA} was elevated (Table 3). Hypotension might stimulate the renin-angiotensin system with subsequent release of angiotensin I into the circulation, which would then be transformed to angiotensin II in the lungs and might stimulate systemic or lung release of PGs. To investigate this possibility six infusions of angiotensin II were given into the inferior vena cava of 4 cats, giving a blood concentration of 0.25-1.25 ng/ml. Assay tissues were also calibrated with angiotensin II. The tissues reacted similarly to equal concentrations of angiotensin II independent of the route of administration. In two cats angiotensin II was infused i.v. twice. Before the second infusion 3 mg/kg indomethacin was given. Angiotensin II caused similar contractions of the tissues before and after PG synthesis inhibition (fig. 15) showing that increased blood level of angiotensin II entering the lungs does not give detectable PG-like activity in carotid arterial blood. Angiotensin II itself might affect

Fig. 14. The effects of elevating left atrial pressure in an anaesthetized, open-chested cat, ventilated at constant volume. (Pressure elevation no 2 in cat no 4). The tracings are: pulmonary arterial pressure (P_{PA}), left atrial pressure (P_{LA}), cardiac output (CO), femoral arterial pressure (P_{FA}), transpulmonary pressure (P_{TP}), and the tensions of the bioassay tissues continuously superfused with arterial blood (10 ml/min) : rat stomach strip (RSS), rat colon (RC) and chick rectum (CR). The assay tissues were calibrated by direct infusion of $PGF_{2\alpha}$. H indicates hyperinflation of the lungs. During the period of P_{LA} elevation, indomethacin was infused i.v.

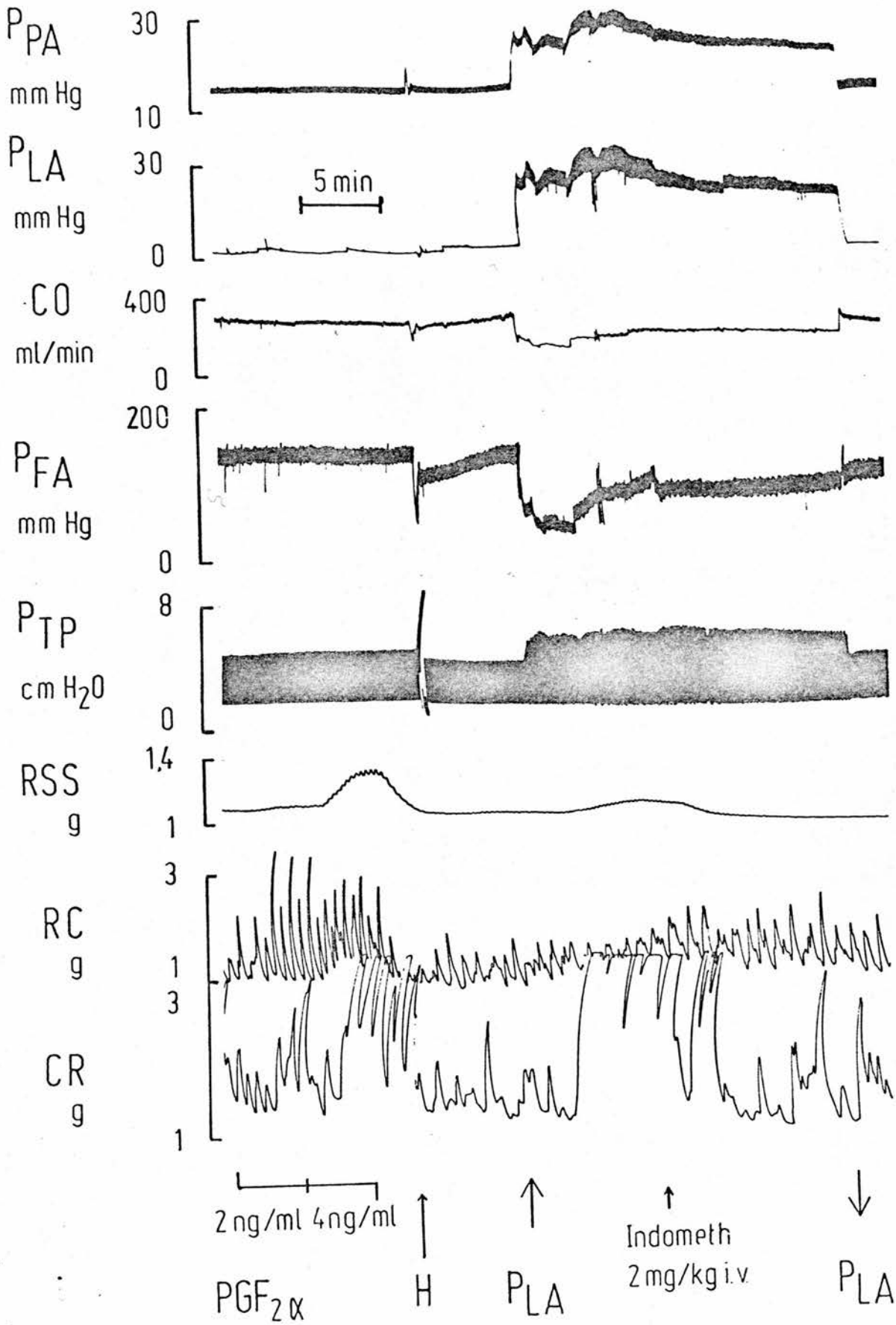

P PA
mm Hg
30
10
P LA
mm Hg
30
0
5 min
CO
ml/min
400
0
P FA
mm Hg
200
0
P TP
cm H2O
8
0
RSS
g
1,4
1
RC
g
3
1
CR
g
3
1
2 ng/ml 4 ng/ml
PGF2α
H
PLA
Indometh
2 mg/kg i.v.
PLA

Fig. 14.

Fig. 15. Effects of infusing angiotensin II i.v. to an anaesthetized cat (cardiac output 400 ml/min) on femoral blood pressure (P_{FA}) and 2 bioassay tissues continuously superfused with arterial blood (10 ml/min): rat stomach strip (RSS) and rat colon (RC) before and after i.v. administration of indomethacin (3 mg/kg) to block PG biosynthesis.

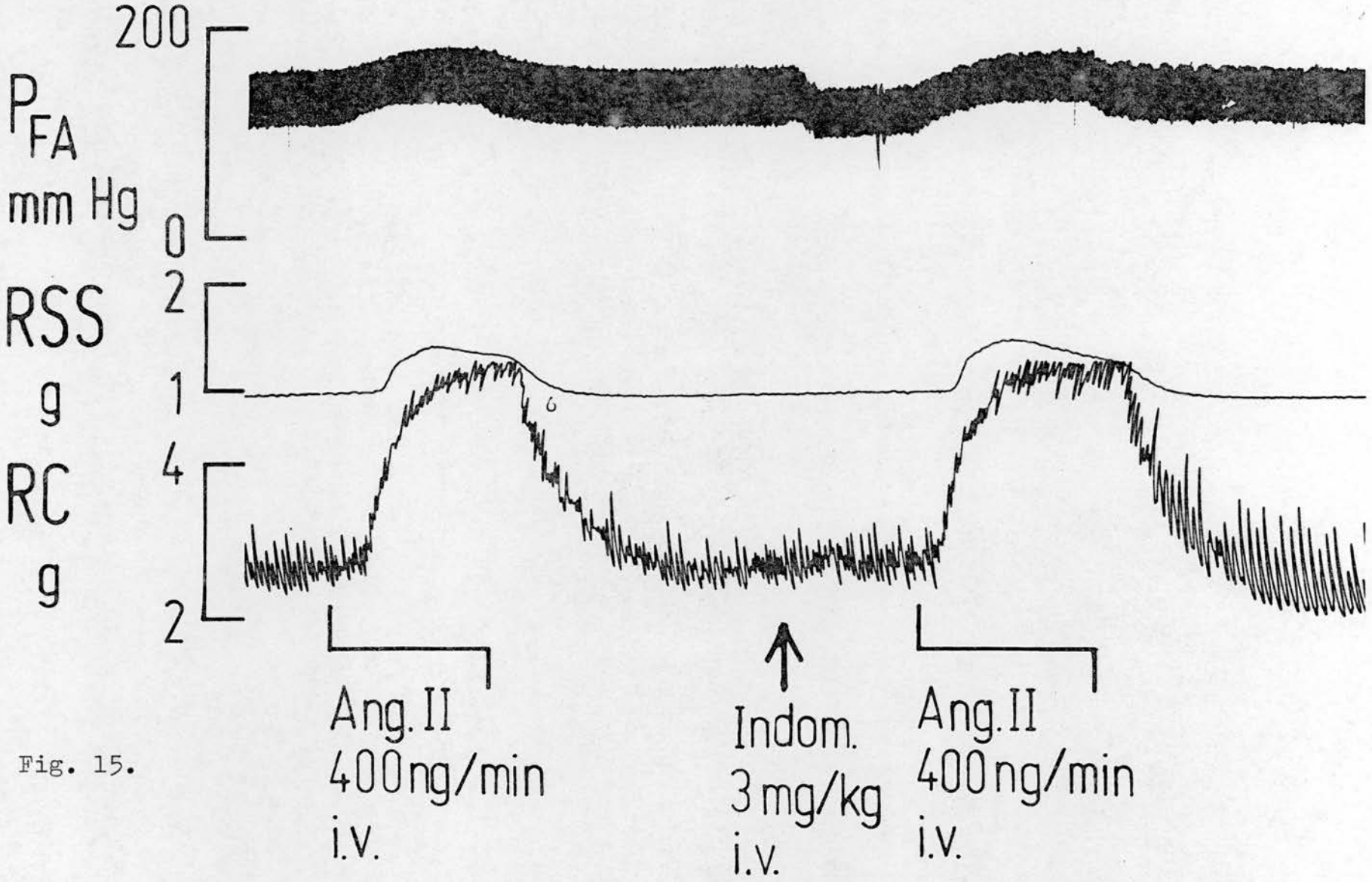

200
P_{FA}
mm Hg
0
2
RSS
g
1
4
RC
g
2
Ang. II
400 ng/min
i.v.
Indom.
3 mg/kg
i.v.
Ang. II
400 ng/min
i.v.

Fig. 15.

the tension of the assay tissues, as the tissues were not blocked against it. To investigate this possibility angiotensin II was infused directly to the assay tissues in concentrations from 0.1 to 1.0 ng/ml. In these doses angiotensin II contracted the RC whereas no or only small contractions of the RSS and CR were observed. This pattern is distinctly different from that of PGE_2 and $PGF_{2\alpha}$ (fig. 16). Consequently angiotensin II cannot be responsible for the observed tissue responses. On the other hand angiotensin II might cause some of the effect observed on the RC and could, together with release of PGE_2, give a picture of $PGF_{2\alpha}$-like activity.

Hematocrit (hct) and Osmolarity

At the onset of each experiment hct was in the range of 27-35 and gradually declined to 15-28 during the experiment, probably due to hemodilution caused by transfusions.

Plasma osmolarity was unchanged at 320-328 m osmol/L throughout each experiment.

Pulmonary Degredation of Prostaglandins

The effect of P_{LA} elevations on pulmonary degredation of prostaglandins was investigated in order to establish whether any systemically-released PGs could have traversed the lungs without being degraded during periods of increased P_{LA}. Three cats (nos. 10-12, Table 4) were pretreated with indomethacin (2mg/kg i.v.) before commencement of the extracorproeal circulation. This dose was considered sufficient to block systemic PG synthesis (Flower, 1974). When indomethacin was infused P_{FA} fell by about 10% and remained at this reduced level. No definite change in mean CO was observed, while P_{LA} and P_{PA} fell slightly.

Fig. 16. Effects of standard doses of PGE_2 , $PGF_{2\alpha}$ and angiotensin II on 3 bioassay tissues superfused with arterial blood (10 ml/min) from an anaesthetized cat : rat stomach strip (RSS), rat colon (RC) and chick rectum (CR).

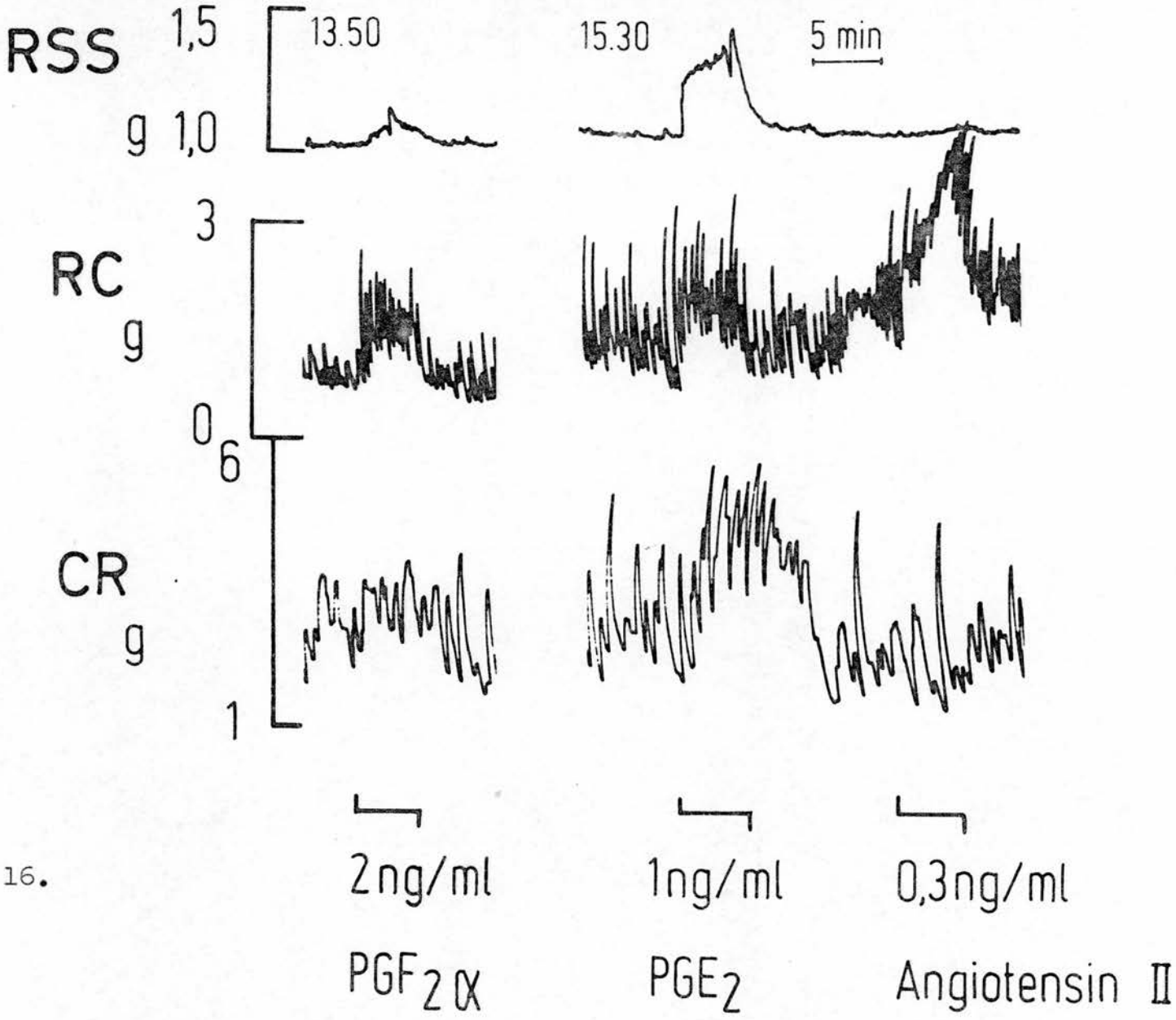

RSS
1,5
g 1,0
13.50
15.30
5 min
3
RC
g
0,6
CR
g
1
2ng/ml
PGF2α
1ng/ml
PGE2
0,3ng/ml
Angiotensin II

Fig. 16.

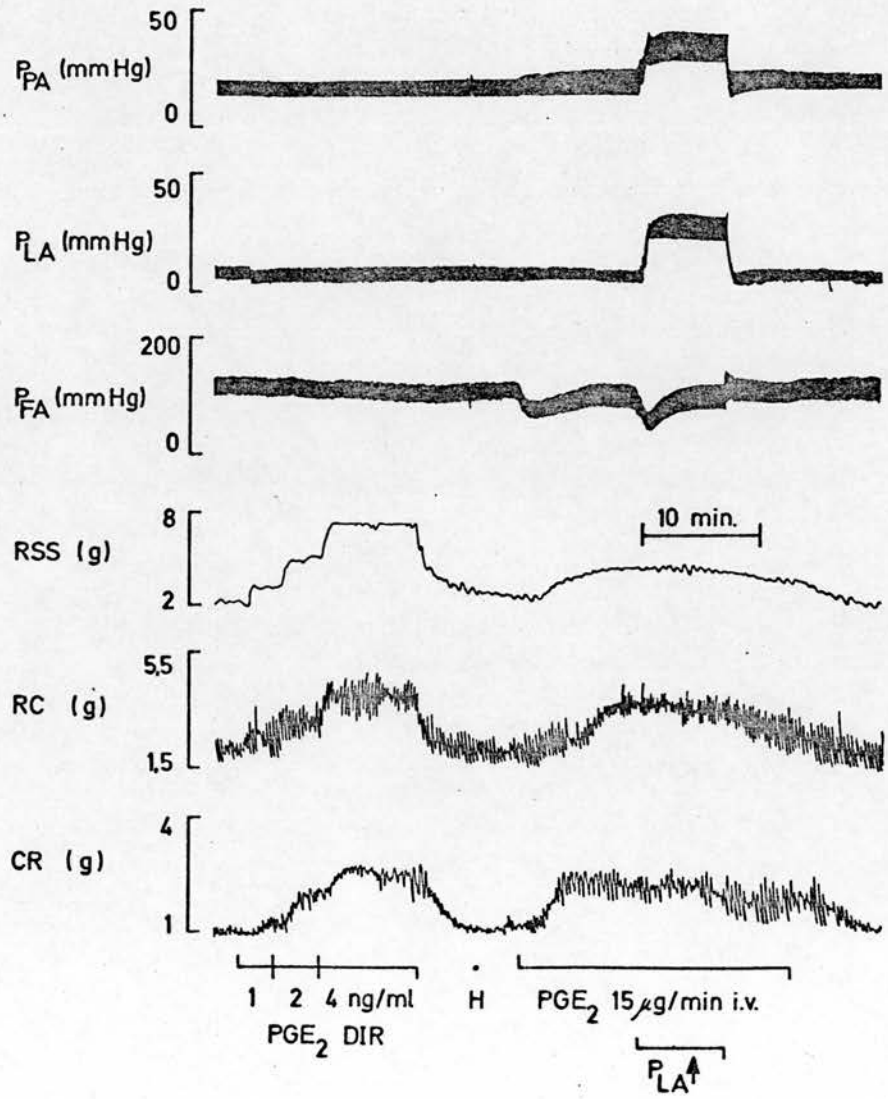
Table 4. Effect of increased P_{LA} on pulmonary degradation of PGE_2.

Cat no.	Cardiac output (ml/min)	PGE_2 infusion i.v. (µg/min)	Activity in art. blood (ng/ml)	P_{LA} (mmHg)	Activity in arterial blood during pressure elevation (ng/ml)	% degradation
10	600	15	2	25	2	92%
11	650	10	3	30	3	81%
12	900	15	2	27	2	88%

When PGE_2 was infused systemically (i.e. into the inferior vena cava close to the heart or into the left atrium) the effect on P_{FA} was biphasic. Within 2 min of the start of infusion P_{FA} fell by about 25% then rose to a value equal to or slightly greater than its pre-infusion level. This increase in P_{FA} was maintained throughout the infusion. CO increased by about 15% during infusion and mean P_{PA} increased by about 20%, thus pulmonary resistance increased during infusion.

Intravenous PG must traverse the lungs and will be metabolized (Ferreira and Vane, 1967a) by enzymes present in the vascular endothelial cells (see p.17). PGE_2 was infused into the inferior vena cava during periods of control vascular pressures in concentrations of 15.4 -25.0 ng/ml/min (Table 4) for periods of up to 23 min, until activity on the assay tissues had reached a plateau. When compared with calibrating doses infused directly to the tissues the activity was equivalent to 2-3 ng/ml PGE_2, giving a value of 81-92% degradation. These values are in agreement with those of Ferreira and Vane (1967a). PGE_2 was infused for up to 25 min. A plateau level of tissue activity was reached and P_{LA} was raised. Tissue activity was unaffected by the elevation of left atrial pressure (8-10 min) or the return to control vascular pressures. One such experiment is shown in fig. 17.

Fig. 17. Vascular and assay tissue effects of an i.v. infusion of PGE_2 (15 µg/min) in an anaesthetized cat. P_{PA}= pulmonary arterial pressure, P_{LA}= left atrial pressure and P_{FA}= systemic arterial pressure. The tensions of the rat stomach strip (RSS), rat colon (RC) and chick rectum (CR) are shown. Tissues were calibrated with direct doses of PGE_2 (DIR). Left atrial pressure (P_{LA}) was raised and returned to control during the infusion of PGE_2. H indicates hyperinflation of the lungs.



SECTION 1 PART 2. RADIOIMMUNOASSAY OF $PGF_{2\alpha}$

RESULTS

The antiserum did not distinguish between the F compounds but showed no cross reaction with PGA, PGB, PGE_1, PGE_2 or the dihydro- or keto-metabolites of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (fig. 20). The RIA method is described fully by Gautvik, Teig, Wiberg, Bronsted and Christoffersen (in preparation). The antibody was produced in chickens. All determinations were carried out in duplicate and differed less than 15%.

Fig. 18 shows the concentration of $PGF_{2\alpha}$ (ng/ml perfusate) in the effluent, measured before and after pressure elevation and induction of pulmonary oedema in one cat lung preparation. The concentration of the main pulmonary metabolite of $PGF_{2\alpha}$, 15-keto, 13-14 dihydro $PGF_{2\alpha}$, was also determined (by Dr. Hans Kindahl, Karolinska Institute, Stockholm).

Table 5 and fig. 19 show the results from 4 experiments. Neither the increase in P_{LA} nor the development of pulmonary oedema in these lungs caused an increase in the $PGF_{2\alpha}$ content of the plasma, which remained constant or, in one cat lung, decreased slightly during perfusion. There is a basal level of PGF_{α} in the perfusate of these lungs of between 0.55 to 3.4 ng/ml before P_{LA} was raised.

Lung tissue content of PGF_{α} was also measured. Seven rabbit lungs were perfused with horse plasma, 3 of which served as controls. Control lungs were perfused for 15-30 min without elevating outflow pressure and frozen during a period of decreasing or stable weight. In 4 lungs P_{LA} was raised to 15 or 20mmHg and maintained until the lungs were grossly oedematous.

Lungs were removed from the perfusion circuit and rapidly immersed

Fig. 18. Changes in plasma PGF and 15-keto,13,14-dihydro $PGF_{2\alpha}$ (measured by RIA) before and after an increase in left atrial pressure (P_{LA}) of 25 mmHg.

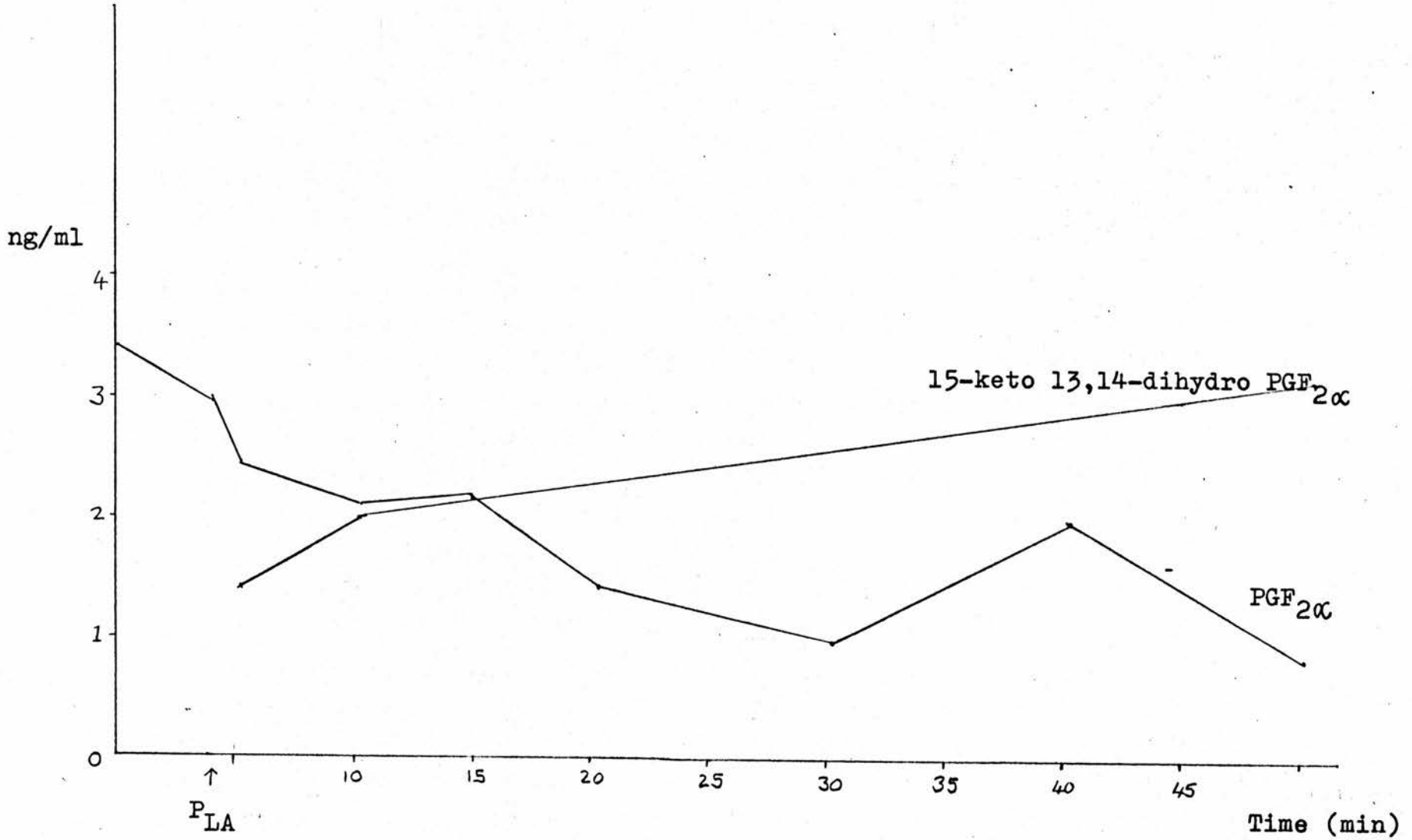


Fig. 19. Shows the changes in the amount of $PGF_{2\alpha}$ (ng/ml) measured in plasma samples from the control value before elevation of left atrial pressure (LAP).

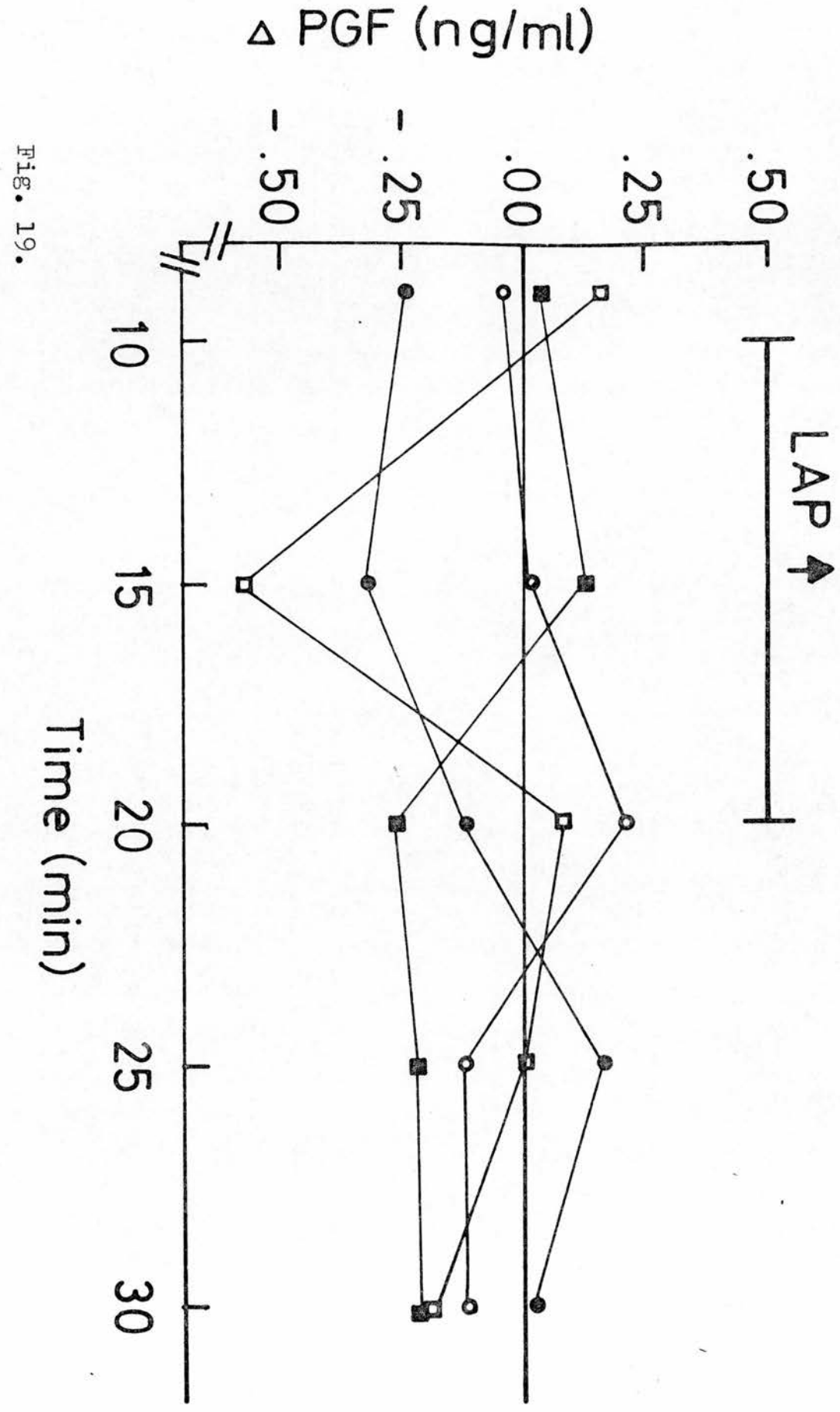


Fig. 19.

TABLE 5. The concentration of $PGF_{2\alpha}$ (ng/ml) in perfusate from 2 cat and 2 rabbit lungs measured by RIA at intervals before and after increasing P_{LA}.

Species	Control		P_{LA} ↑	TIME (min)										
				1	5	10	15	20	25	30	35	40	45	60
cat	1.75	1.6		0.7	1.0	0.65	1.75	0.8	0.7	1.3	1.7			
cat	3.4	2.95		2.43	2.1	2.18	1.35		0.93		2.05		0.83	
rabbit	2.05	3.11		2.6	2.98	2.48		2.48		3.58		2.49		2.0
rabbit	0.75	0.55		0.65	0.6	0.95	0.85		0.75		1.3		1.1	

in liquid DDM to halt PG synthesis.

The values for tissue content of PGF_{α} are shown in table 6a. Tissue content of PGF_{α} was not significantly different in oedematous lungs from that measured in the control situation ($p>.429$, Mann Whitney U-test one sided). These values are slightly higher than the basal levels for $PGF_{2\alpha}$ (8ng/g wet tissue weight) reported in rabbit lung tissue by Karim (1968).

The concentration of PGF_{α} in tracheal froth is shown in table 6b. Duplicate measurements were made in samples of tracheal exudate from 7 rabbit lungs by the same method used for plasma PGF_{α} determination. Values found were between 1.0ng/ml and 5.76 ng/ml (mean 2.73). Alveolar fluid in oedema probably represents the outflow from the interstitium to the alveoli of fluid filtered from the exchange vessels and as such is difficult to obtain a control value for, since sampling of interstitial fluid from normal lungs is impossible. However, the values for PGF_{α} in tracheal froth are not different from those in the perfusate.

Thus in hydrostatic pulmonary oedema, PGF_{α} concentration is not increased in perfusate from isolated lungs, or in total tissue. Concentration in tracheal fluid is not significantly higher than that of plasma.

TABLE 6a. Tissue content of PGF_{α} (ng/g wet tissue weight)

CONTROL	OEDEMA
13.75	17.5
13.75	13.75
16.25	11.25
	43.75

TABLE 6b. Tracheal fluid content of PGF_{α} (ng/ml). (n)= no. of samples

5.21	(2)
1.75	(2)
1.0	(1)
5.76	(1)
2.25	(3)
2.75	(3)
1.78	(3)

Fig. 20. amount of unlabled compound needed for 50% displacement of ^{3}H-PG-$F_{2\alpha}$ from rabbit anti-$PGF_{2\alpha}$-serum.

prostaglandin	$F_{2\alpha}$	0.3×10^{-9}g
prostaglandin	$F_{1\alpha}$	0.3×10^{-9}g
15 keto prostaglandin	$F_{2\alpha}$	$> 100 \times 10^{-9}$g
15-keto, 13,14 dihydro prostaglandin	$F_{1\alpha}$	$> 100 \times 10^{-9}$g
15-keto, 13,14 dihydro prostaglandin	$F_{2\alpha}$	$\approx 30 \times 10^{-9}$g
prostaglandin	E_1	$> 100 \times 10^{-9}$g
prostaglandin	E_2	$> 100 \times 10^{-9}$g
prostaglandin	A_1	$> 100 \times 10^{-9}$g
prostaglandin	B_1	$> 100 \times 10^{-9}$g
prostaglandin	B_2	$> 100 \times 10^{-9}$g

Determined in 2 different assays where the results differed by less than 20%.

SECTION 2. RELEASE OF PROSTAGLANDINS DURING PULMONARY MICROEMBOLISM

RESULTS

Effects of Collagen Infusions

a) Pulmonary and Vascular Effects

In total, 43 collagen infusions were given i.v. to 10 cats, each animal receiving 3-7 infusions. Collagen infusion caused a marked lung response as judged from the increase in P_{PI} (figs. 21 and 23) during, and for a few minutes after the infusion. It has previously been shown (Vaage, Bö and Hognestad, 1974) that pulmonary arterial pressure (P_{PA}) also increases in parallel with increase in P_{PI}, although P_{PA} was not measured in the experiments reported here. The size of the lung response varied between cats, although in individual cats the response to the first 4-6 collagen infusions was similar.

During infusions there was a temporary decrease in systemic blood pressure (P_{FA}) in all but 5 cases in 3 animals where no reduction in P_{FA} occurred, although the lung responses were present.

b) Release of Prostaglandin-like Substances (PGLS)

Three to four minutes after the start of each collagen infusion, the bioassay tissues contracted, indicating release of PGLS (figs. 21, 22, 23, 24 and 25). This release was assayed as 0.5 - 2 ng/ml of PGE_2 or $PGF_{2\alpha}$ - like substances. However, the pattern of contractions could usually not be exactly matched by PGE_2 or $PGF_{2\alpha}$. When RbA was included in the assay, collagen infusions caused contractions of this tissue (figs. 21, 22 and 23) demonstrating release of 'rabbit aorta contracting substance' (RCS, see p.47). PGLS were detected during every collagen infusion where a lung response was present.

During 5 of the collagen infusions either the RC or the CR did

not contract. However, in these cases the non-responding tissue was also insensitive to calibrating doses of 2-4 ng/ml PGE_2 and $PGF_{2\alpha}$.

Abolition of Assay Tissue Activity

Airway responses decline and finally disappear with repeated collagen infusions (Vaage, Bø and Hognestad, 1974). It was, therefore, of interest to see if tissue activity also disappeared when the lung responses were exhausted. However, the additional trauma to the animal of the extracorporeal circulation during repeated infusions prevented the use of bioassay throughout the experiment. Therefore two cats without extracorporeal circulation were given infusions of collagen at 30 min intervals until neither a lung response nor platelet aggregation could be elicited. This procedure reduced the number of circulating platelets to 25-30 per cent of the initial value. At this stage blood superfusion of the bioassay tissues was started, and each animal was given 3 more collagen infusions, to which neither contractions of the assay tissues nor lung reactions occurred. A further two animals were given an initial infusion of collagen which evoked the usual lung response and release of PGLS. Indomethacin (5 mg/kg) was then infused i.v. This caused a maintained decrease in P_{FA} of 15 and 20 mmHg. Subsequent collagen infusions caused neither lung reactions nor contractions of the bioassay tissues.

Platelet Aggregation ex vivo and in vivo

In 3 experiments one initial i.v. infusion of collagen (0.2 ml/min) was given (in vivo aggregation), then platelet aggregation was induced ex vivo by a smaller dose of collagen (0.05 ml/min) infused directly into the extracorporeal circulation upstream to the assay

tissues. In vivo and ex vivo platelet aggregation caused similar contractions of the assay tissues, except the RC which showed a much smaller contraction during ex vivo aggregation (fig. 23).

Release of Kinins and Angiotensin II

The assay tissues were not blocked against the action of kinins and angiotensin II. Control experiments were therefore performed to investigate whether release of these substances might be responsible for part or all of the tissue contraction pattern observed. In two experiments a cat jejunum (CJ) was included as an assay organ and all tissues were calibrated with bradykinin as well as PGs. Bradykinin calibrations in the concentrations used (0.5-4 ng/ml), contracted the CJ, but had little or no effect on the RSS, RC and CR (fig. 24). No significant contraction of the CJ was elicited by i.v. collagen infusions. Release of kinins cannot be responsible for the contraction pattern of the assay tissues observed during collagen infusions.

A fall in systemic blood pressure was observed during most of the collagen infusions. Angiotensin II might be released secondary to such reductions in blood pressure. However, during a total of 5 collagen infusions in 3 animals, there was no change in P_{FA} although the usual lung responses and contractions of the bioassay tissues were observed (fig. 21). Also calibrating doses of angiotensin II given directly to the assay tissues (0.1-0.6 ng/ml) showed that the pattern of tissue contractions occurring during induced platelet aggregation could not be matched by standard doses of angiotensin II (fig. 25). These doses of angiotensin II, however, caused contraction of the RC. Release of angiotensin II might therefore be responsible for at least part of the contraction of the RC observed during collagen infusions

where systemic hypotension occurred.

Fig. 21. Effects of inducing intravascular platelet aggregation by collagen infusions in an anaesthetized cat ventilated at constant volume. The following bioassay tissues were continuously superfused with arterial blood (10 ml/min) from the cat: rabbit aorta (RbA), rat stomach strip (RSS), rat colon (RC) and chick rectum (CR). TP is the tracheal pressure and FAP femoral arterial pressure. The tissues were calibrated with infusions of PGE_2 and PGF_2 directly into the superfusing blood. Isometric tensionoof the tissues is shown.

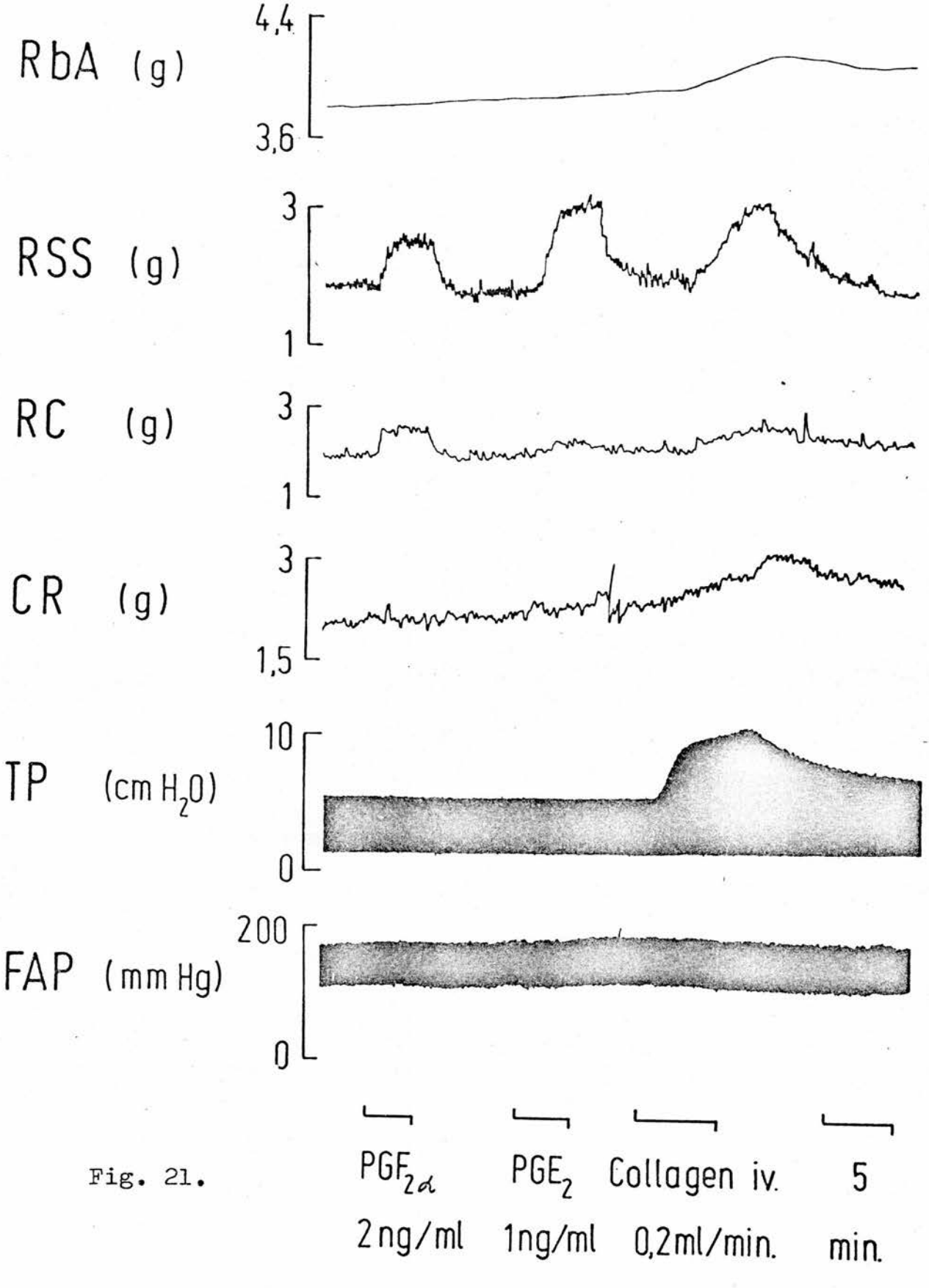

RbA (g)
4,4
3,6
RSS (g)
3
1
RC (g)
3
1
CR (g)
3
1,5
TP (cm H2O)
10
0
FAP (mm Hg)
200
0
PGF2α
2ng/ml
PGE2
1ng/ml
Collagen iv.
0,2ml/min.
5
min.

Fig. 21.

Fig. 22. Effects of 4 consecutive i.v. collagen infusions (0.2 ml/min) to an anaesthetized cat. The bioassay tissues are as in fig. 21. In this figure the scale of the RbA is zero-suppressed. The asterisk in the tracing of the RC indicates that the tissue touched its surrounding tube and showed a false contraction.

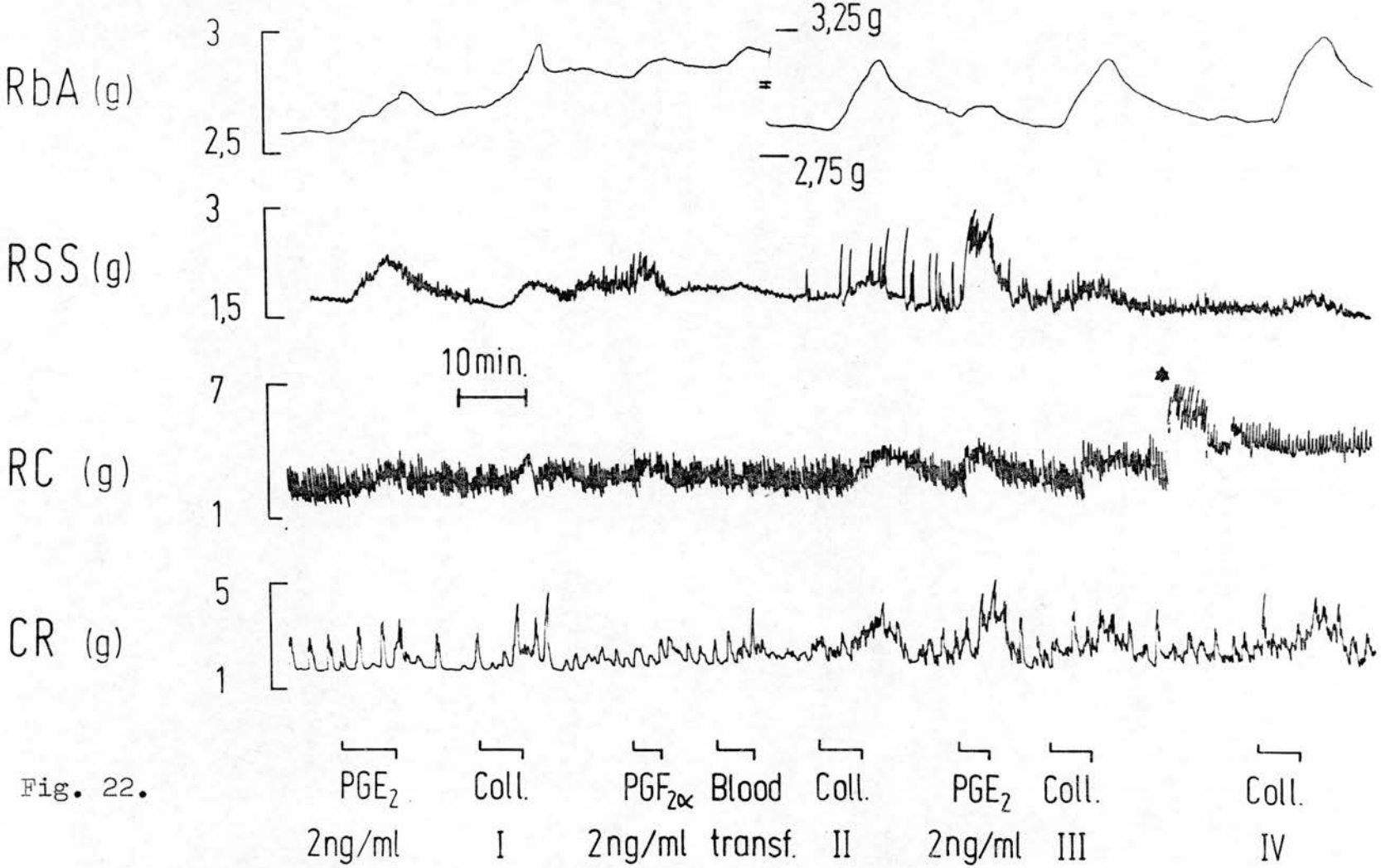

RbA (g)
3
2,5
3,25 g
2,75 g
RSS (g)
3
1,5
10 min.
RC (g)
7
1
CR (g)
5
1
PGE2
2ng/ml
Coll.
I
PGF2α
2ng/ml
Blood
transf.
Coll.
II
PGE2
2ng/ml
Coll.
III
Coll.
IV

Fig. 22.

Fig. 23. Effects of inducing intravascular platelet aggregation by collagen infusions in an anaesthetized cat ventilated at constant volume. TP=tracheal pressure, FAP= femoral arterial pressure, RbA= rabbit aorta, RSS= rat stomach strip, RC= rat colon and CR= chick rectum. The tissues were calibrated with standards of PGE_2 and $PGF_{2\alpha}$. Collagen was infused either i.v. or directly into the blood bathing the assay tissues.

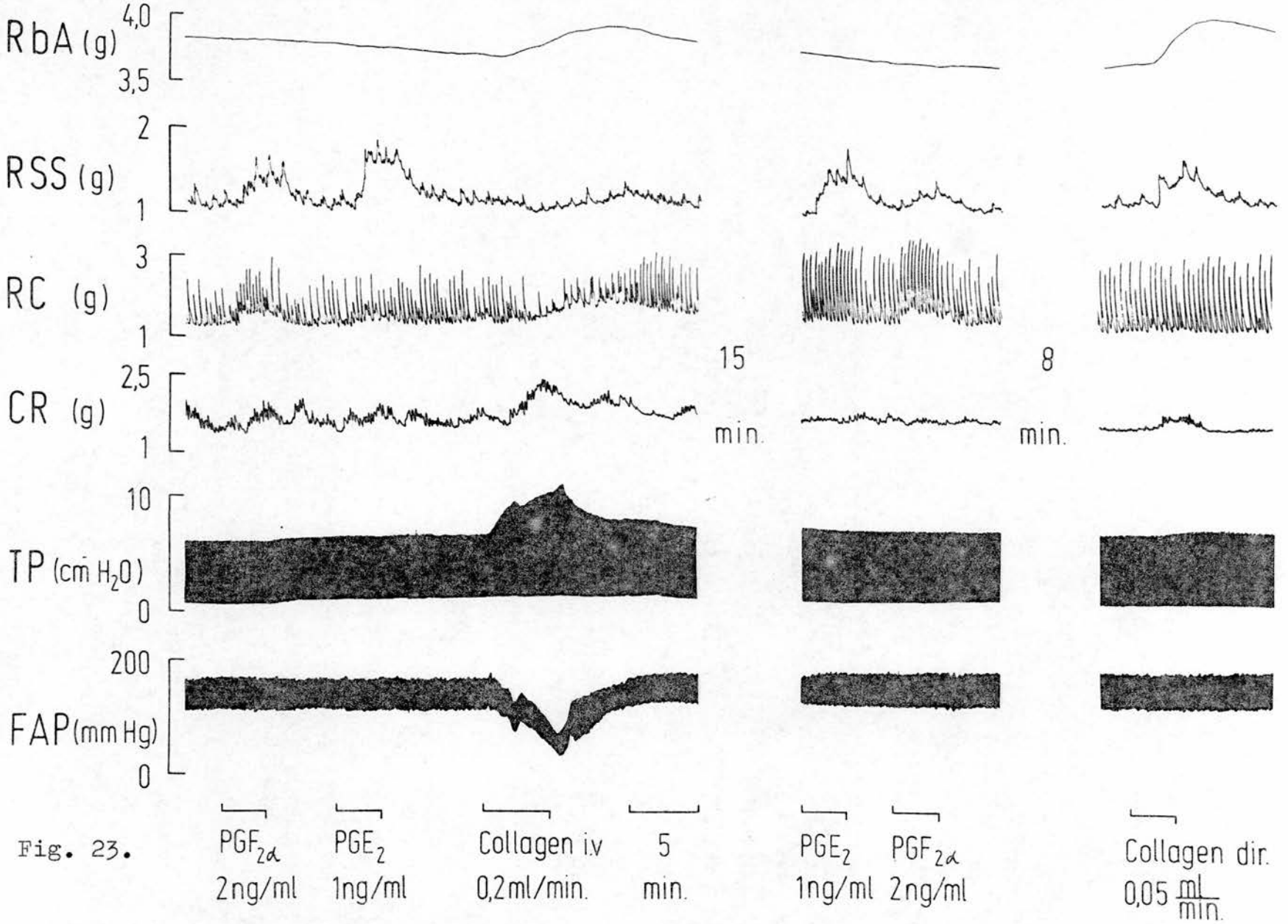

RbA (g)
4,0
3,5
RSS (g)
2
1
RC (g)
3
1
CR (g)
2,5
1
TP (cm H2O)
10
0
FAP (mm Hg)
200
0
15
min.
8
min.
PGF2α
2ng/ml
PGE2
1ng/ml
Collagen i.v
0,2ml/min.
5
min.
PGE2
1ng/ml
PGF2α
2ng/ml
Collagen dir.
0,05 ml/min.

Fig. 23.

Fig. 24. Effects of inducing intravascular platelet aggregation by consecutive i.v. infusions of collagen in an anaesthetized cat on femoral arterial pressure (FAP), and on the following bioassay tissues continuously superfused with arterial blood (10ml/min) from the animal: cat jejunum (CJ), rat stomach strip (RSS), rat colon (RC) and chick rectum (CR). The tissues were calibrated regularly by infusions of PGE_2 and $PGF_{2\alpha}$ and bradykinin (BK) directly into the superfusing blood. Isometric tension of the tissues is shown. At the arrow, the sensitivity of the CR was reduced electronically by 50%.

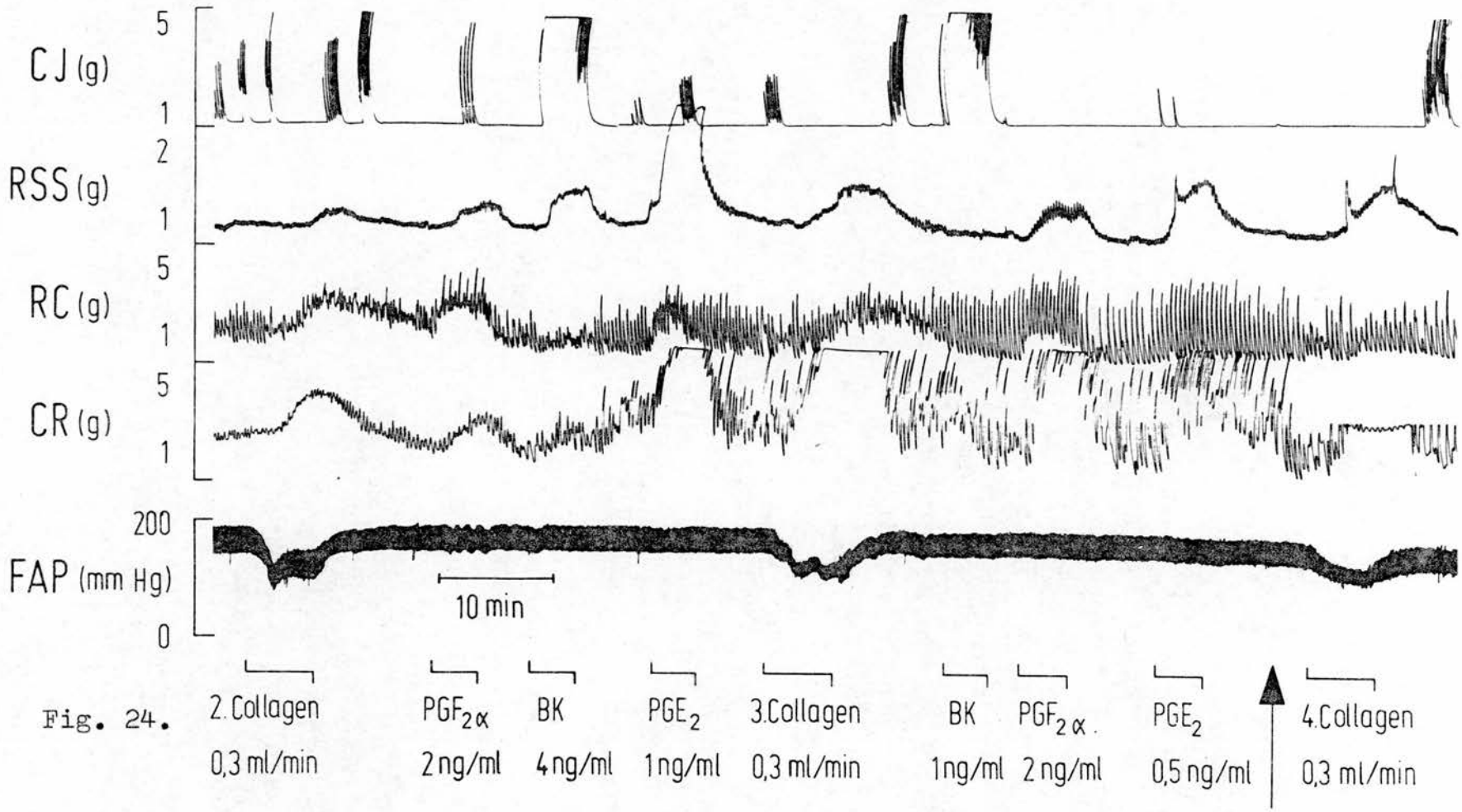

CJ (g)
5
1
RSS (g)
2
1
RC (g)
5
1
CR (g)
5
1
FAP (mm Hg)
200
0
10 min
2. Collagen
0,3 ml/min
PGF2α
2 ng/ml
BK
4 ng/ml
PGE2
1 ng/ml
3. Collagen
0,3 ml/min
BK
1 ng/ml
PGF2α
2 ng/ml
PGE2
0,5 ng/ml
4. Collagen
0,3 ml/min

Fig. 24.

Fig. 25. Effects of infusing collagen to an anaesthetized cat on the following bioassay tissues superfused with 10 ml/min of carotid arterial blood : RSS= rat stomach strip, RC= rat colon and CR= chick rectum. These effects are compared with those of calibrating doses of PGE_2, $PGF_{2\alpha}$ and angiotensin II (Agt. II) infused directly into the superfusing blood. In this particular experiment systemic blood pressure fell from 115 to 95 mmHg during collagen infusion.

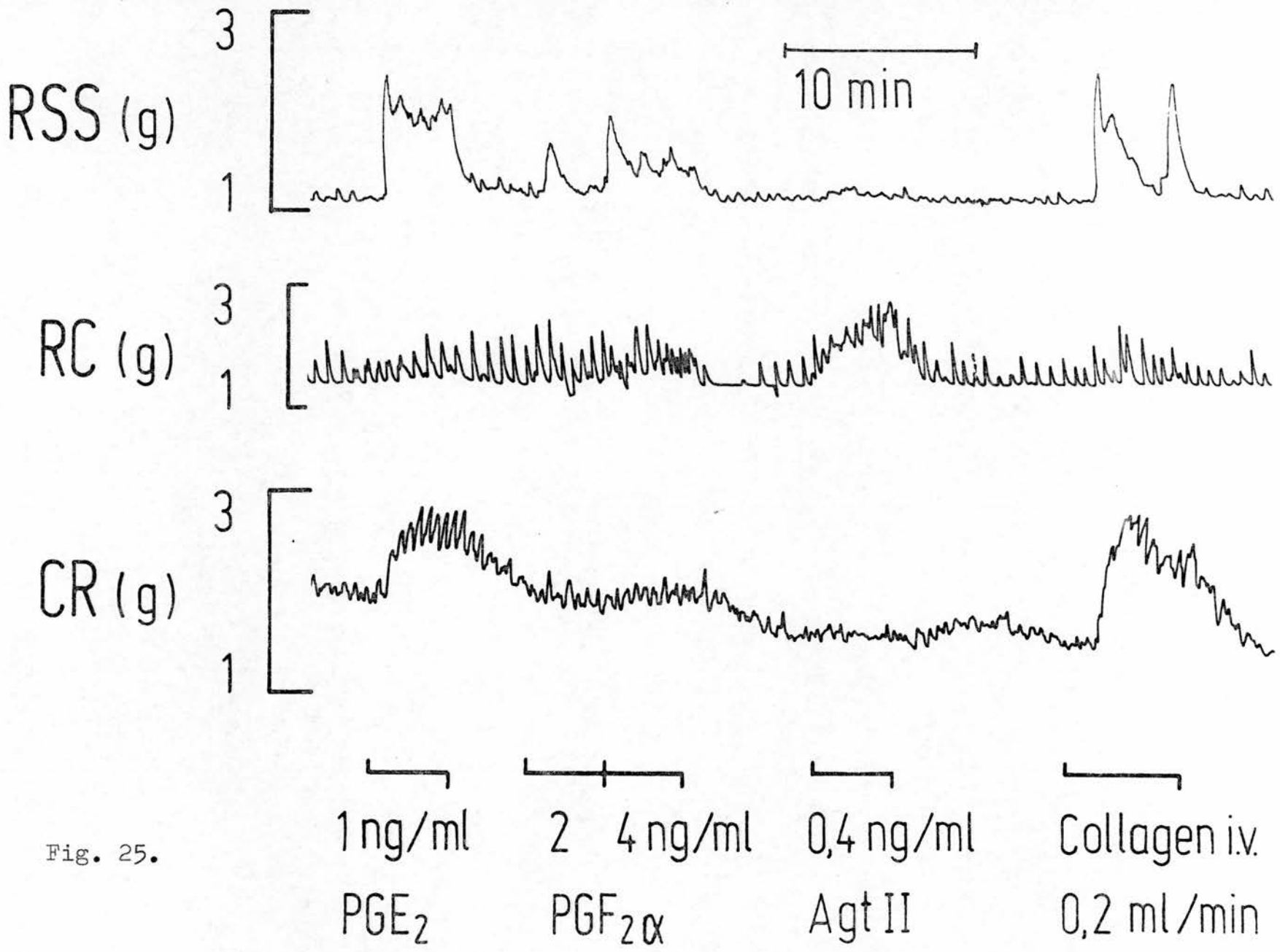

RSS (g)
3
1
10 min
RC (g)
3
1
CR (g)
3
1
1 ng/ml
PGE2
2 4 ng/ml
PGF2α
0,4 ng/ml
Agt II
Collagen i.v.
0,2 ml/min

Fig. 25.

SECTION 3. HYDROSTATIC PULMONARY OEDEMA AND CHANGES IN C_L

RESULTS

Lungs Fixed by Perfusion with Glutaraldehyde Solution

Samples of tissue from lungs fixed by glutaraldehyde perfusion were taken from three regions as described. Oedematous lungs showed a vertical gradient in the degree of oedema: accumulation of oedema fluid was most marked in the dependent parts of the lungs. Many sections were therefore required to ensure representative sampling and tissue samples from the three lung regions: upper, middle and lower were usually taken.

Nineteen experiments were performed. Random samples of between 10 to 33 sections per experiment were taken from the upper, middle and lower lung lobes in 15 experiments and from two lung regions in the four others, and the sections were examined in the LM at magnifications of x100 and x250.

In appearance the cell nuclei stained dark blue and the cytoplasm paler blue. Alveoli were observed in an irregular mesh formation and alveolar sacs and ducts were seen. Larger vessels and airways were sometimes seen. Filled alveoli were stained an even pale blue colour, as were the contents of the venules and arterioles. Alveoli were classified as either filled or empty. However, in contrast to the results of Staub, Nagano and Pearce (1967) some partly-filled alveoli were seen, a concave meniscus stretching over the opening of the alveolus from the alveolar sac. These were classified as filled alveoli.

Quantification of Alveolar Oedema

An attempt was made to quantify alveolar oedema by inspection. A Leitz graticule with two crosslines at 90° to one another was inserted into the eyepiece of the Leitz orthoplan microscope. Random areas of the sections were examined at magnifications of x100 to x250 and the number of filled or non-filled alveoli scored. The results are shown in Table 7a. Between 400 and 800 alveoli were scored in each experiment and the percent of filled alveoli calculated. Experiments where weight increase was 10g showed a fall in C_L.

Lungs Fixed by Rapid Freezing

In 22 experiments lung tissue samples were taken from the upper region of the lower or middle lobes - i.e. approximately on the horizontal level of the hilus - and from the lower part of the lower lobes about 0.5-1 cm from the lobe tip. No samples were taken from the upper lobes as they were often distorted in the clamping and freezing procedure. Tissue from these two regions was cut and embedded in epon (p. 67), sectioned by microtome and stained as described (p.68). A random sample of 6-12 sections from both areas were examined under the light microscope.

In experiment 2, only epon-embedded sections were examined and no samples were taken for paraffin-embedding. In experiments 3-23 tissue samples were embedded in paraffin for sectioning: in all but 3 experiments samples were taken from the upper part of the lower lobe, while in the remaining 3 lungs samples from the lower part only were embedded. In 8 experiments blocks from both areas were embedded and in 2 experiments (17 and 18) two blocks from the same area were used.

Initially sections were cut from the surface of the prepared block, then serial sections were cut between 1 and 2 mm deeper in the

block. Two or three horizontal areas of 1 cm^2 from each block were then available for quantification of oedema enabling a large area of the lung to be seen.

Sections were examined at magnifications of x25 to x250 under the light microscope. In appearance the nuclei of cells stained purple while plasma stained pink. The freeze-drying process causes ice crystals to sublimate leaving "strings" of coagulated protein which stained pink and could be clearly seen in the lumen of the larger arteries and veins (fig. 26). At the pleural edges of the lungs, where freezing was fastest and the ice crystals formed smallest, the protein "strings" were close (fig. 27a) while deeper in the lungs larger ice crystals were formed and the "strings" more clearly visible (fig. 27). Lymphatics lay in the perivascular interstitium of the arterioles and were often very distended in lungs where several grams of fluid had been filtered (fig. 28). They were narrow or not visible in control lungs, although occasionally large lymphatics were observed (figs. 29,30,31 and 32). In control lungs the capillaries in the alveolar walls were of a narrower diameter than in congested lungs (fig. 33). Interstitial areas around the larger vessels and bronchi were stained purple and the tissue was fairly dense. In control lungs the interstitium in that area was usually a very narrow perivascular or peribronchial cuff although distended interstitium was observed in one interstitial area in lung 2 (fig.31). Alveoli were an open meshwork and had no contents.

In test lungs where fluid filtration had occurred, interstitial oedema was visible as large cuffs around the arterioles or bronchi. As described in the introduction, the pulmonary arteries run close to the bronchi, while the veins lie in the area between two arterial trees

(fig. 34). Distended interstitium consisted of interstitial tissue (collagen matrix as described in the introduction) filled with the exudate from the exchange vessels. In many experiments cells were seen in the interstitium, often clustered in the area between the arteriole and bronchiole (see figs. 35, 36 and 37) and were probably leucocytes. Large vessels were observed which did not possess cuffs (while others in the vicinity of airways did) and these were considered to be veins (see fig. 34).

Alveolar flooding was often observed in the alveoli in the area close to perivascular and peribronchial cuffs (fig. 38). Otherwise flooding was often patchy and a picture of filled or non-filled alveoli was seen (figs 39 and 40). In areas where the alveolar sac was sectioned transversely a pattern of partly-filled alveoli radiating from the alveolar sac could sometimes be seen (figs. 41-44). There was a concave meniscus over the entrance to flooded alveoli and in severe oedema, often over the entrance to the alveolar sac. Alveolar ducts were usually empty even in quite severe flooding (fig. 45). In very severe oedema bronchiolar flooding was very occasionally observed (fig. 46). Alveolar flooding was often seen to occur in the alveoli in the areas surrounding the larger arterioles and bronchioles in lobes where little flooding otherwise existed(fig.47).

Quantification of Alveolar Oedema

Quantitative morphometry was based on the method of Weibel (1963) as used by Caldini, Leith and Brennan (1975). A 25 point reticle (integrating eyepiece 1 of Zeiss, according to the design of Hennig 1958) was inserted in the ocular of the Leitz "orthoplan" microscope and the epon and paraffin embedded sections from rapidly frozen lungs

were examined at a magnification of x100 or x250. For each field of 25 points the number which overlay filled and empty alveoli (and alveolar walls) were scored. Points overlying alveolar ducts or sacs were not scored, nor were arterioles, bronchioles, perivascular or peribronchial interstitium or lymphatics.

Between 1861 and 6602 points overlying alveoli were counted. In most experiments over 2500 points were counted to improve accuracy of the results (Caldini, Leith and Brennan, 1975). The fraction of points over filled alveoli was calculated and expressed as a percentage of the total counted (Table 7b). As this is a ratio, and can be compared between experiments, it was not necessary to correct for section thickness (Weibel, 1963). However, since filled alveoli have a smaller volume than non filled alveoli this method may underestimate the proportion of filled alveoli. All lungs were fixed at end expiratory pressure. Table 7b shows the results from 22 experiments in rapidly frozen lungs where the percentage of filled alveoli of the total number of alveoli counted is shown. The number of points counted is also shown.

Fig. 48 shows the percentage of alveolar flooding and weight at the point of fixation for each lung. The percentage change in P_{TP} from the initial P_{TP} (30sec after raising P_{LA}) to P_{TP} at the point of fixation is also plotted. The results show that at low weight gains (that is 3.5 — 10g) there is some alveolar flooding: a progressive increase in flooding of up to 10% of alveoli occurs. Also at weight increases of 10-12g, where the 2^{o} fall can be expected to start, there is an increase in the amount of flooding (10-20%) and in lungs where the 2^{o} fall had clearly occurred there is a sharp increase in the amount of alveolar flooding (48-88% at 15-17.9g).

Point Counting Method for the Quantification of Interstitial Oedema

The major accumulation of interstitial liquid during oedema formation was around the larger arterioles and bronchioles (diameter about 100 μ to 1 mm), and was visible both within and outside dilated lymphatics (figs.49, 50 and 51). Photomicrographs were taken with a 35 mm camera attached to the microscope at magnifications of x5 to x20 of the areas of the sections where these vessels and airways were present. The slides were projected onto a screen of (40 x 60 cm) with a grid of points 0.5 cm apart (method of Bland, personal communication to Dr. G. Nicolaysen). Sometimes slides were projected onto graph paper (40 x 60 cm) and the outlines of the arterioles, bronchioles and interstitial cuffs were drawn, and the number of points 0.5 cm apart counted. A micrometer scale photomicrograph of the same magnification was also projected.

The number of points within vessels and the number in the perivascular cuffs were counted and a ratio of cuff to vessel lumen was obtained. Mainly arterioles and their perivascular cuffs were counted since they possessed the largest interstitial areas (figs. 52 and 53). Peribronchial cuffs were often smaller (figs. 54 and 55) and only those with large cuffs were counted. Bronchial arterioles when seen had large cuffs (figs. 56 and 57). In these areas where vessels and bronchi shared interstitium the area round the vessels was point-counted taking a demarcation line for the perivascular cuff by extrapolation if necessary. The ratio of cuff to vessel (bronchus) sizes for each experiment is shown in Table 2 along with the number of vessels (or broncni) counted per experiment

TABLE 7a.

Experiment no.	Weight at fixation (grams)	Percentage filled alveoli	No. slides
5	control	0	13
6	control	0	10
7	control	0	12
13	control	0	13
18	control	0	12
10	3.4	2	19
15	3.4	0	30
9	6.1	13	10
12	6.5	1	11
1	7.5	1	26
11	8.8	2	19
17	9.4	5	15
8	9.8	14	21
19	14.8	24	12
4	16.3	21	33
2	16.4	50	10
14	17.1	59	10
3	19.4	54	32
16	20.0	57	15

Weight (in grams) and percentage of alveolar flooding in lungs fixed by gluteraldehyde perfusion.

TABLE 7b

Percentage Alveolar Flooding in Rapidly-Frozen Lungs

Experiment number	Weight at Fixation	Precentage Alveolar flooding	Number of Alveoli counted
2	0	0	1816
18	0	0	6173
19	0	0	2529
3	2.7	2.21	5242
4	4.0	6.53	2877
5	5.0	21.3	4036
10	5.5	9.16	3397
23	7.5	9.81	2936
11	9.0	9.1	2757
12	9.3	10.13	2102
14	9.7	16.03	5041
9	10.2	9.4	2093
6	10.6	13.85	3387
7	11.0	28.3	4405
16	11.2	23.7	3078
17	11.2	8.1	3815
8	11.7	20.0	3140
20	13.4	76.0	2457
15	15.0	47.9	4184
21	15.0	85.16	6602
22	17.9	48.83	2511

TABLE 8.

Experiment no.	Ratio cuff.vessel	no. vessels counted
2	0.5	8
18	0.2	6
19	0.21	5
3	3.0	3
4	3.2	4
5	2.9	3
10	5.25	3
23	2.84	5
11	3.7	4
12	3.5	3
14	2.9	4
9	4.1	2
6	3.8	4
7	3.5	3
16	2.4	4
17	4.9	2
8	3.5	4
20	2.5	5
15	3.1	2
21	2.0	4
22	2.86	4

Ratio of cuff to vessel size (from point counting method) in rapidly-frozen lungs, and the number of vessels or airways counted per experiment.

Fig. 26 shows a section of a pulmonary vein (experiment 3). Large protein "strands" have been formed during the freezing procedure, and can be clearly seen.

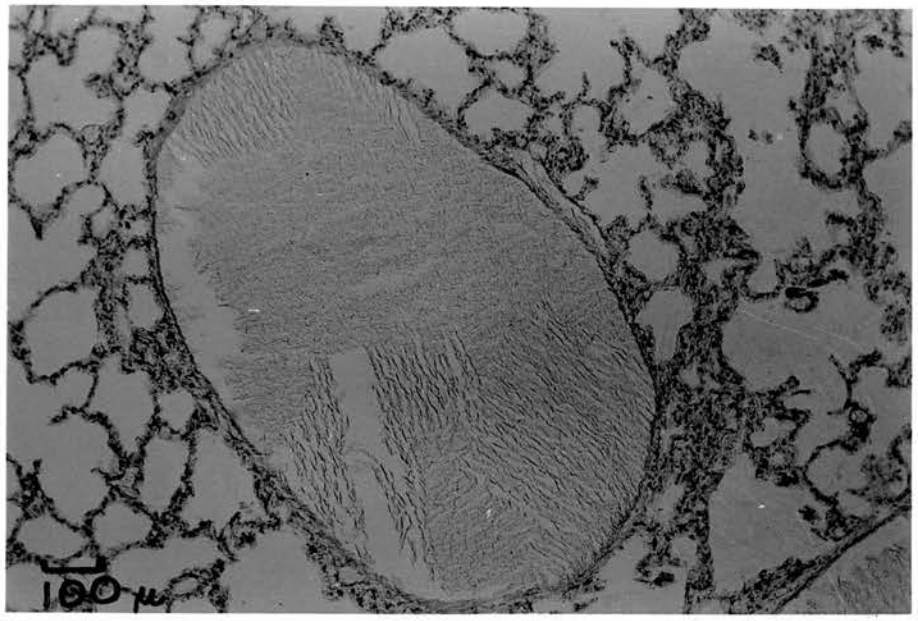


Fig. 27 Experiment 23, lower lobe. A transverse section of the lobe specimen, beginning from the septum, is shown. Contents of the peripheral venules and arterioles are homogeneous, while those of deeper vessels show clear strands of protein.

Fig. 27.

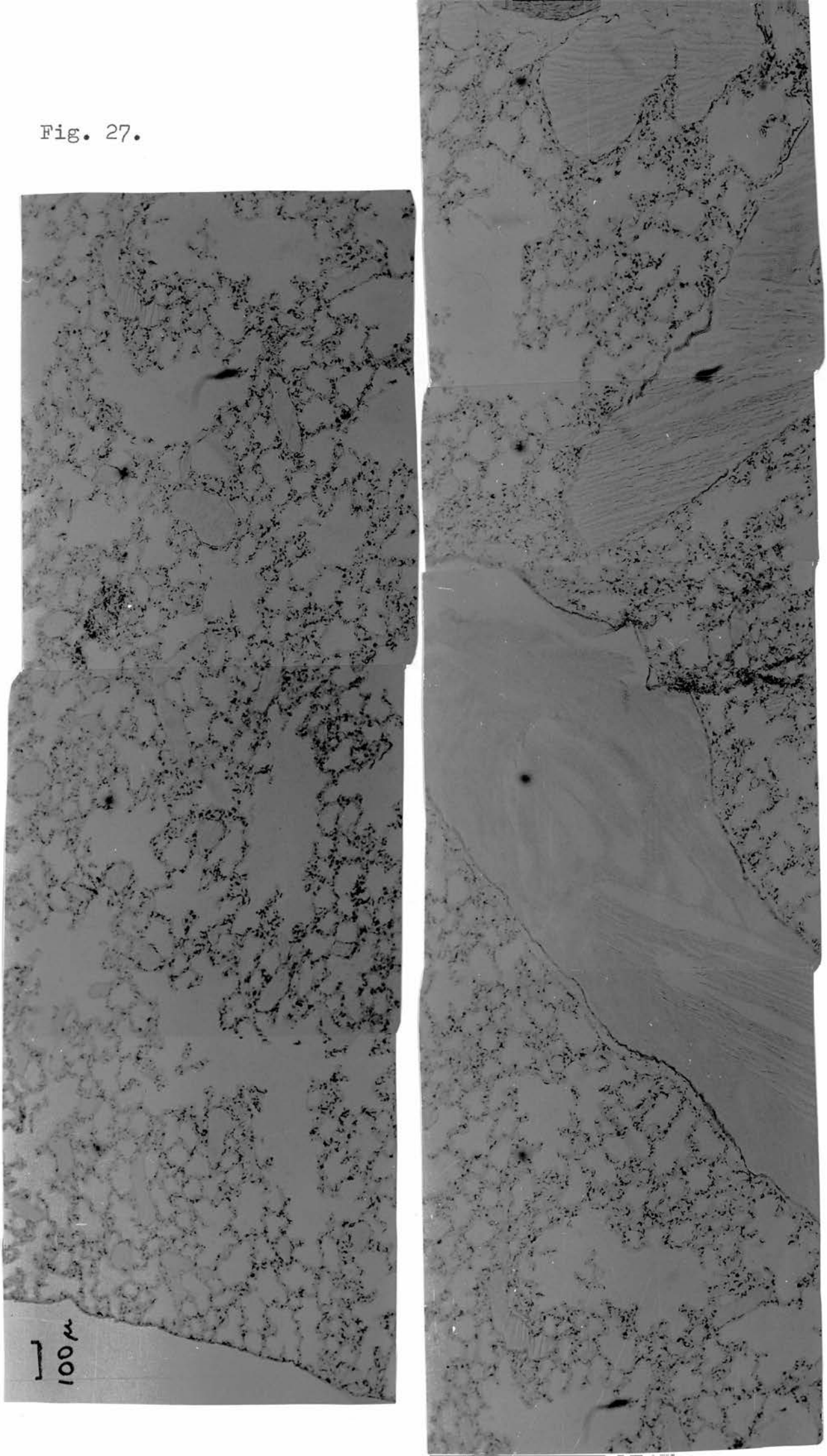
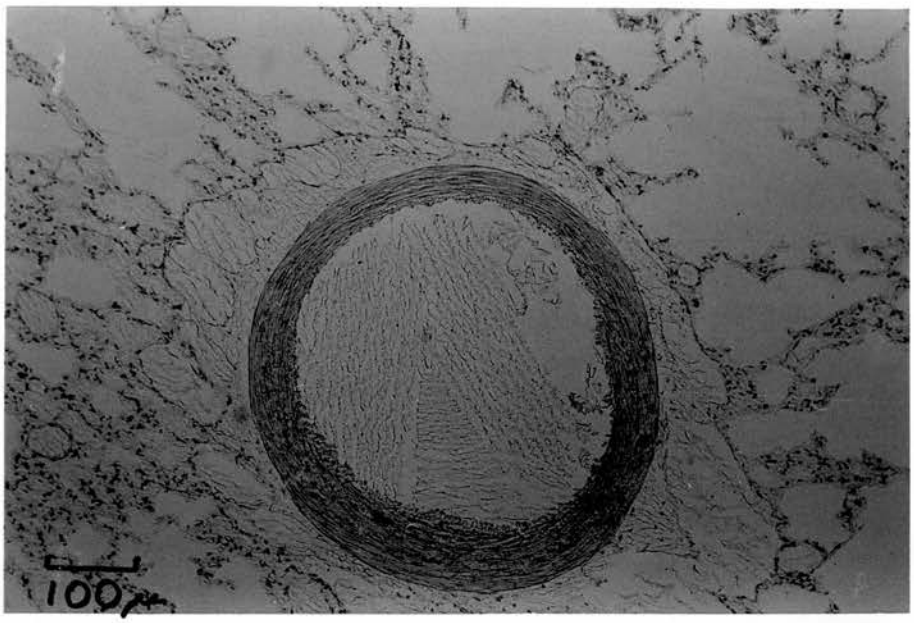
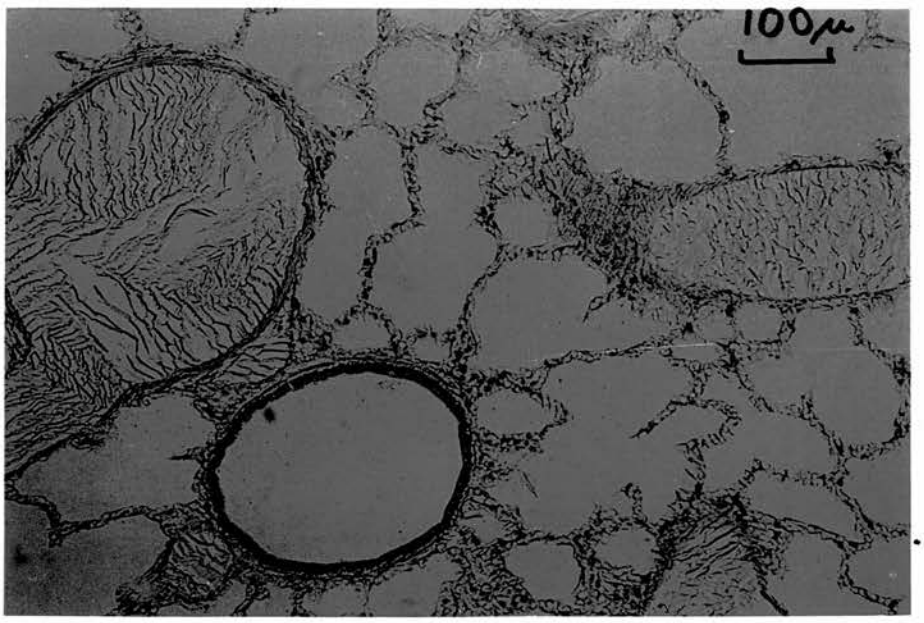


Fig. 28 Experiment 4. A perivascular cuff and distended lymphatics are seen in this experiment, where 4 grams of fluid was filtered.



Figs 29 and 30. Experiment 2. No perivascular or peribronchial oedema was visible in these control lungs, although some filled lymphatics are seen.



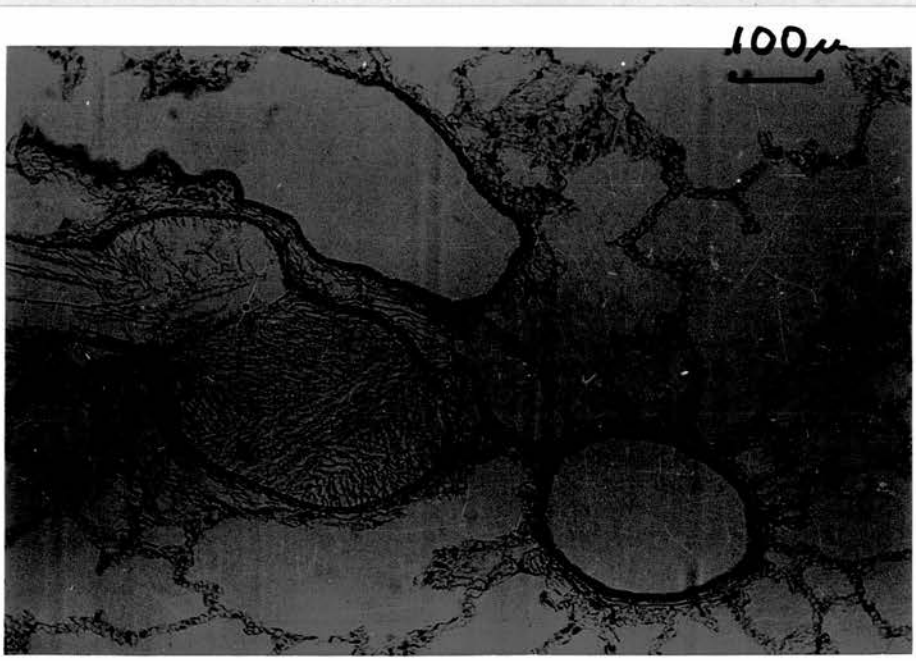


Fig. 31. Considerable perivascular oedema is seen. L Lymphatics are distended, and flooded alveoli are absent. (Experiment 2).

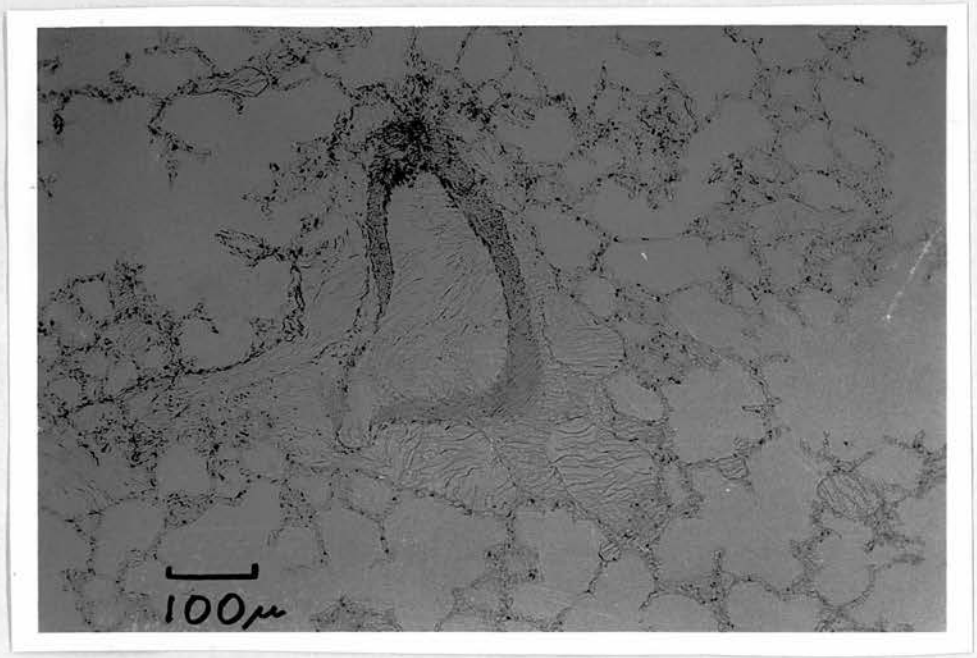
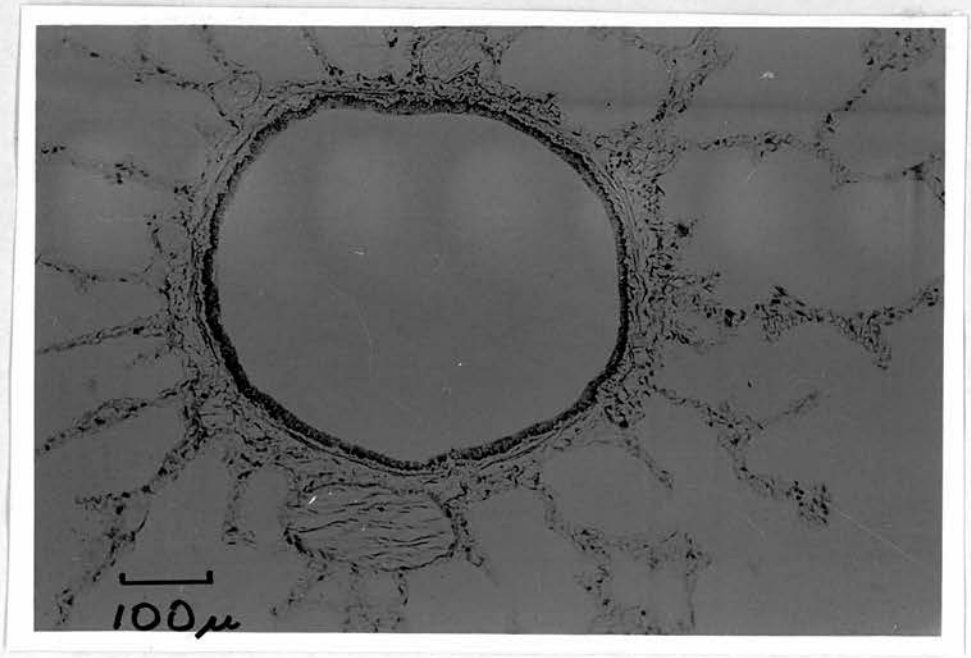


Fig. 32. No peribronchial oedema is visible, and the alveoli are empty.



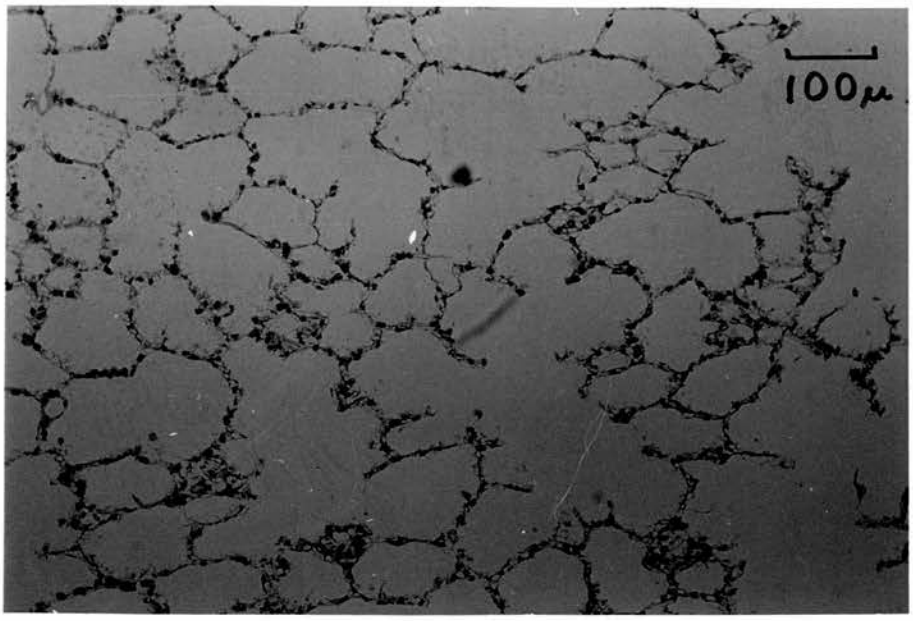


Fig. 33. Experiment 18,control. No contents in the alveoli and alveolar walls are narrow when compared with experiments with raised P_{LA}.

Fig. 34. Relative positions of pulmonary arteries, veins and bronchi.

PA=pulmonary artery PV=pulmonary vein Br=bronchus

(on facing page)

Fig. 34.

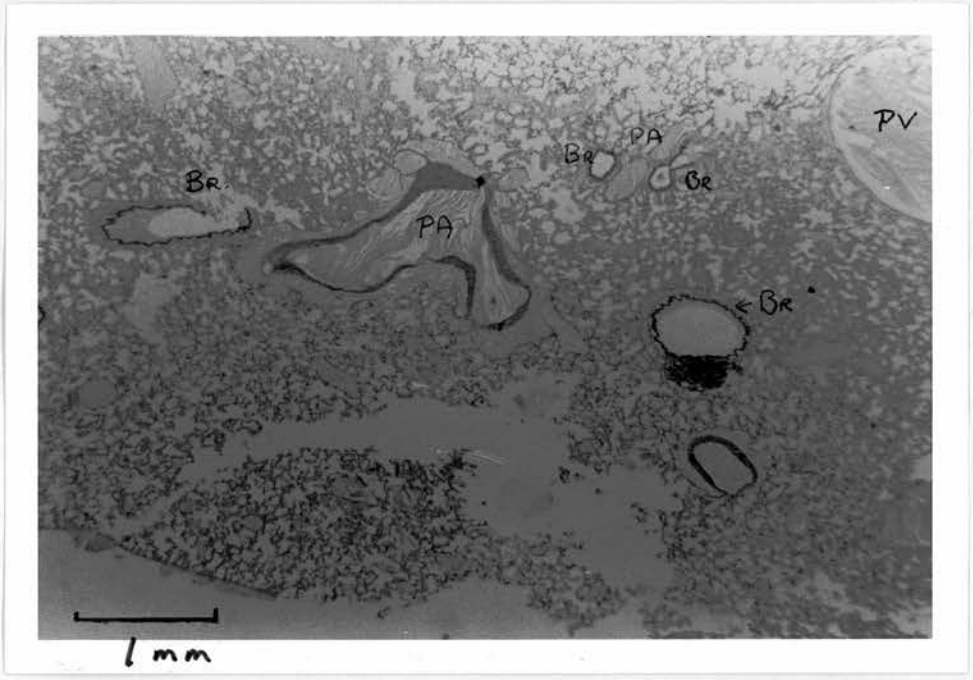


Fig. 35

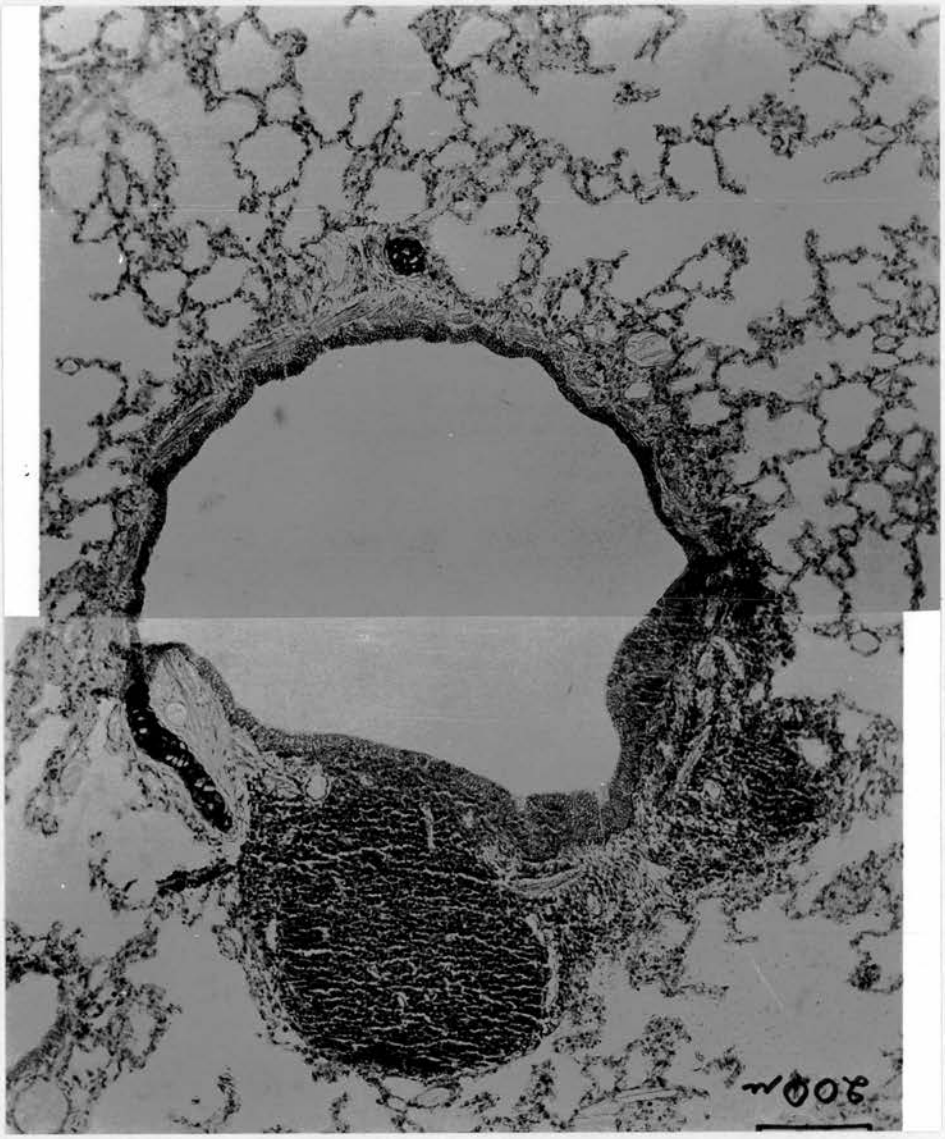
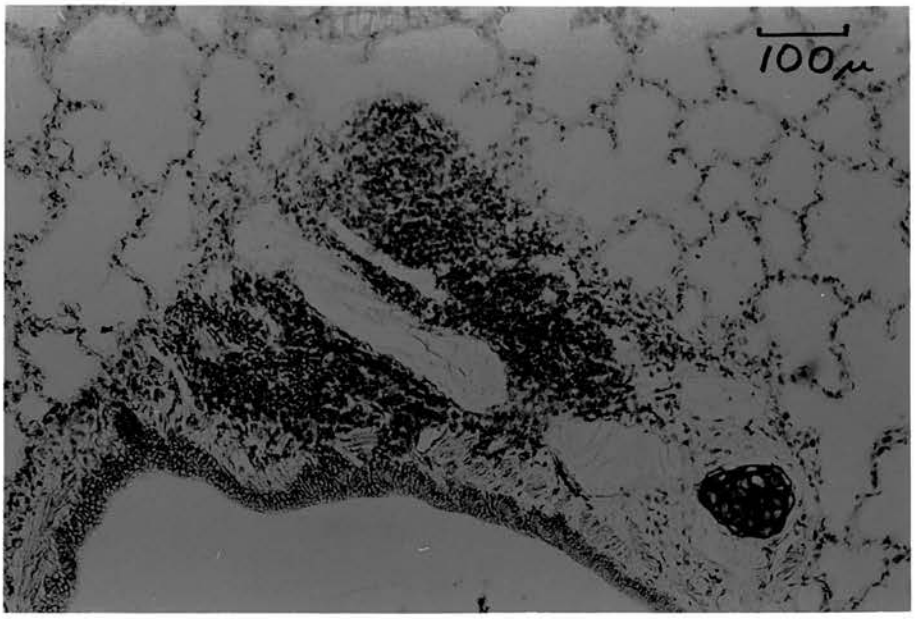


Fig. 36



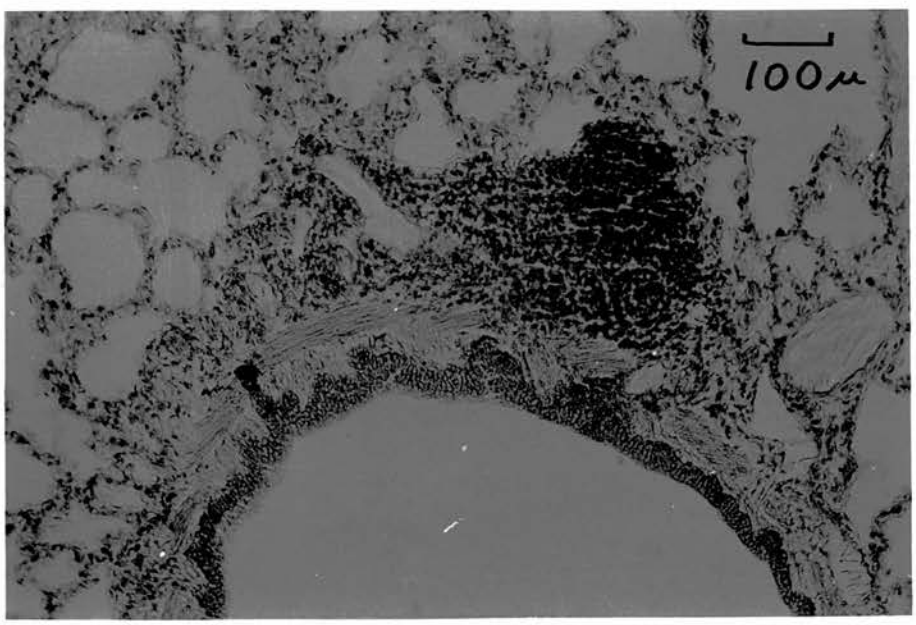


Fig. 35 and 36. Leucocytes in the peribronchial area in experiment 3.

Fig. 37. Leucocytes close to a pulmonary vessel in experiment 16.

Fig. 37.

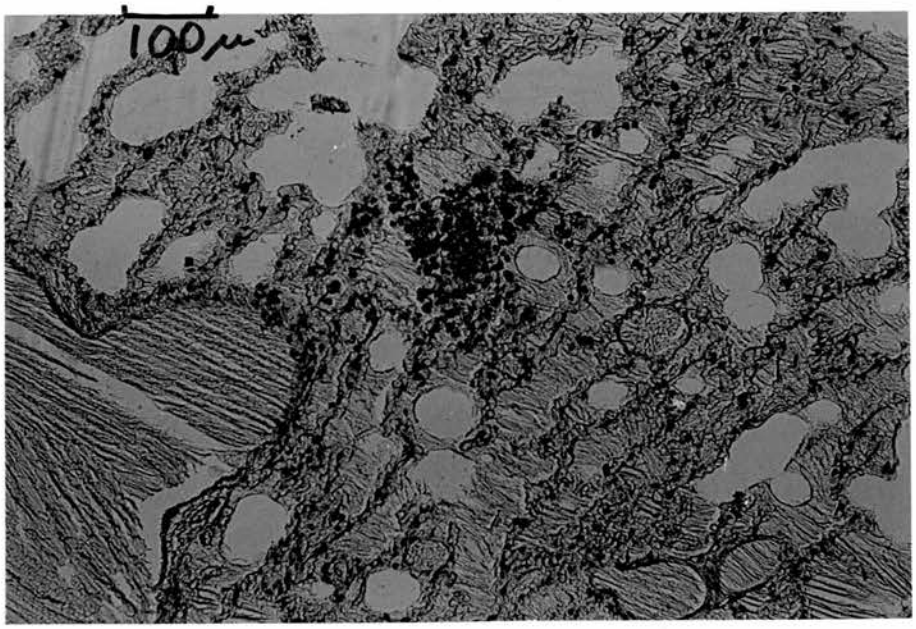


Fig. 38. Alveolar oedema in the area near a vessel with perivascular oedema. (Experiment 4).

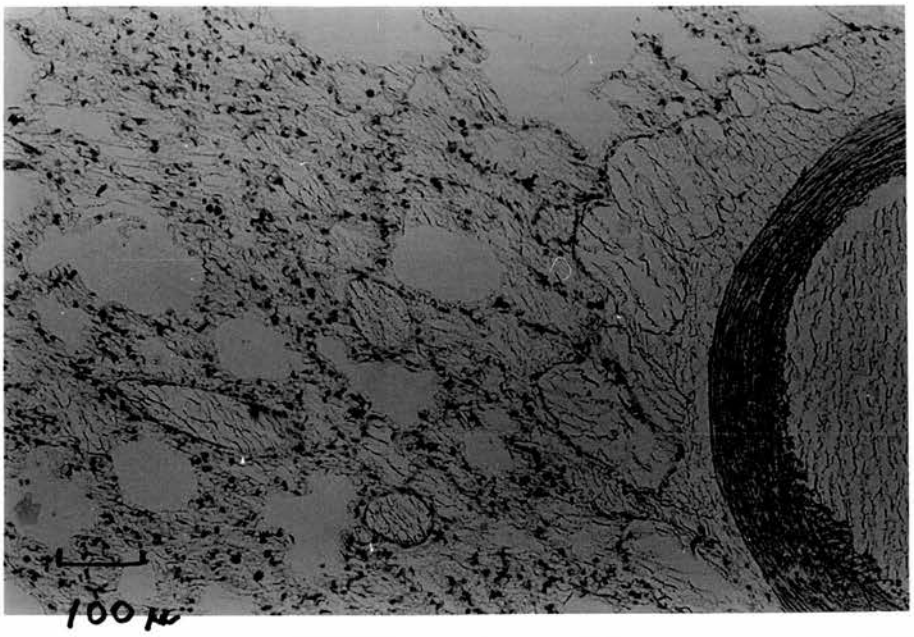
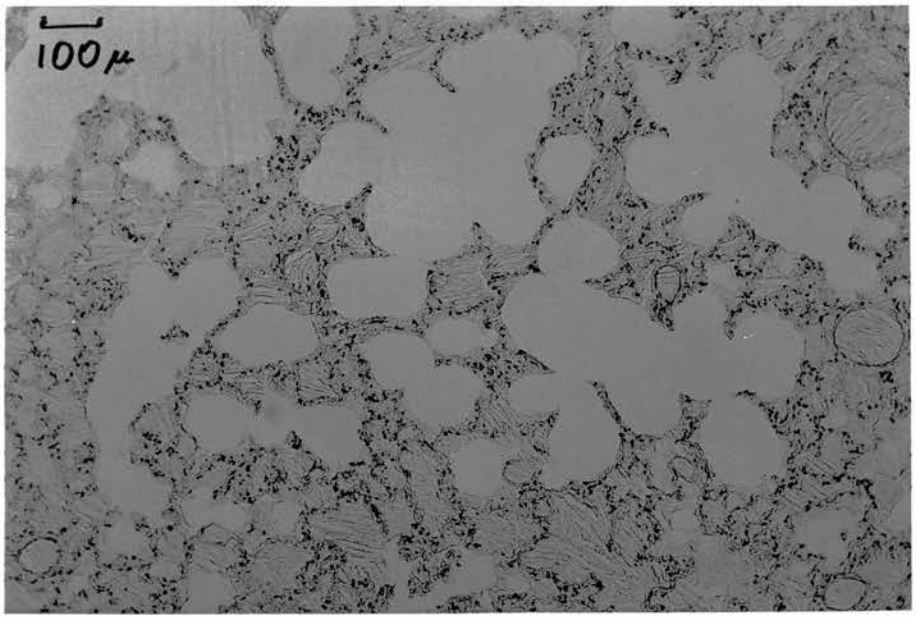


Fig. 39. Alveolar flooding is "patchy". Flooded areas are interspersed with partly-filled or empty alveoli. The partly-filled alvaoli are seen radiating from a single alveolar sac. (Experiment 15).



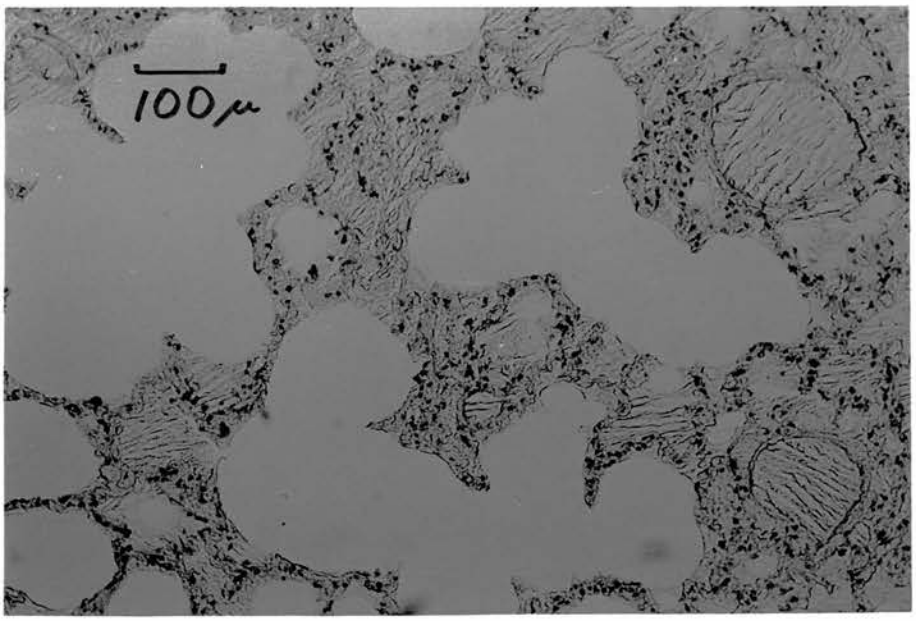


Fig. 40. Alveolar flooding is not uniform in this area. Also note the presence of bubbles of trapped gas (arrow). (Experiment 15).

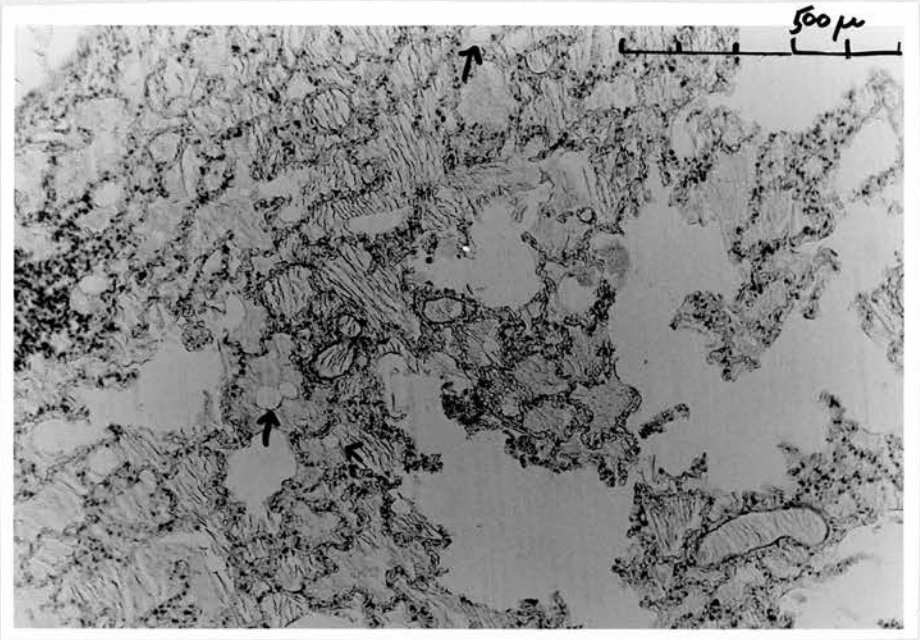


Fig 41. Experiment 23. Area near septum showing filled and partly-filled alveoli. Note filled alveoli with a concave meniscus over the entrance from the (empty) alveolar sac.

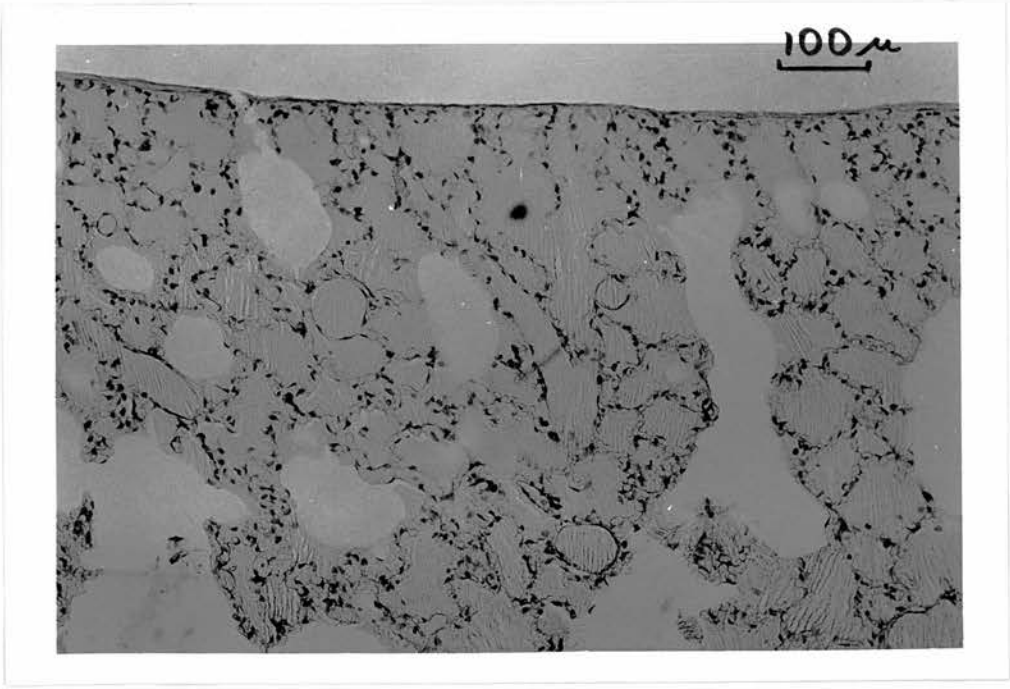
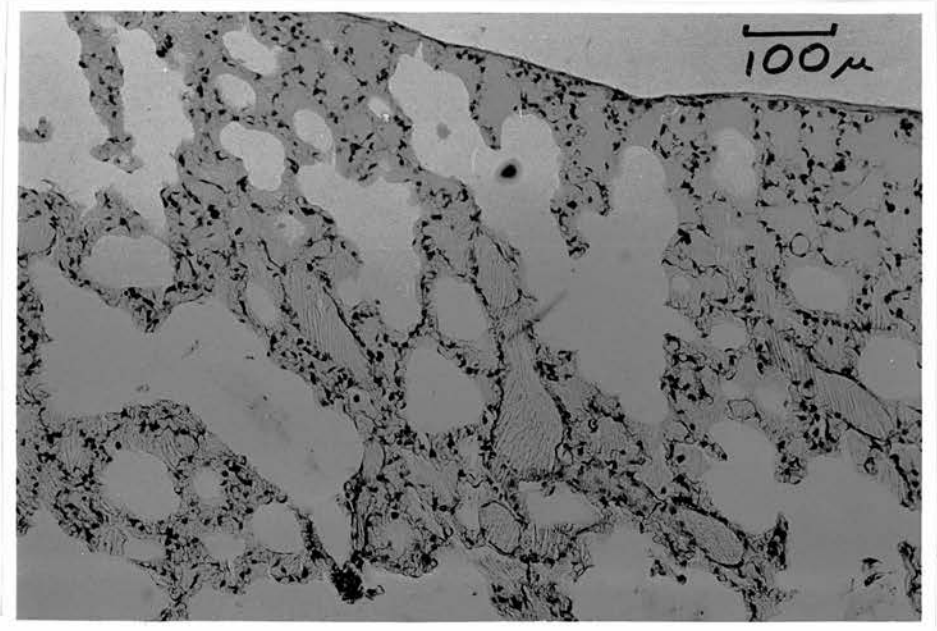
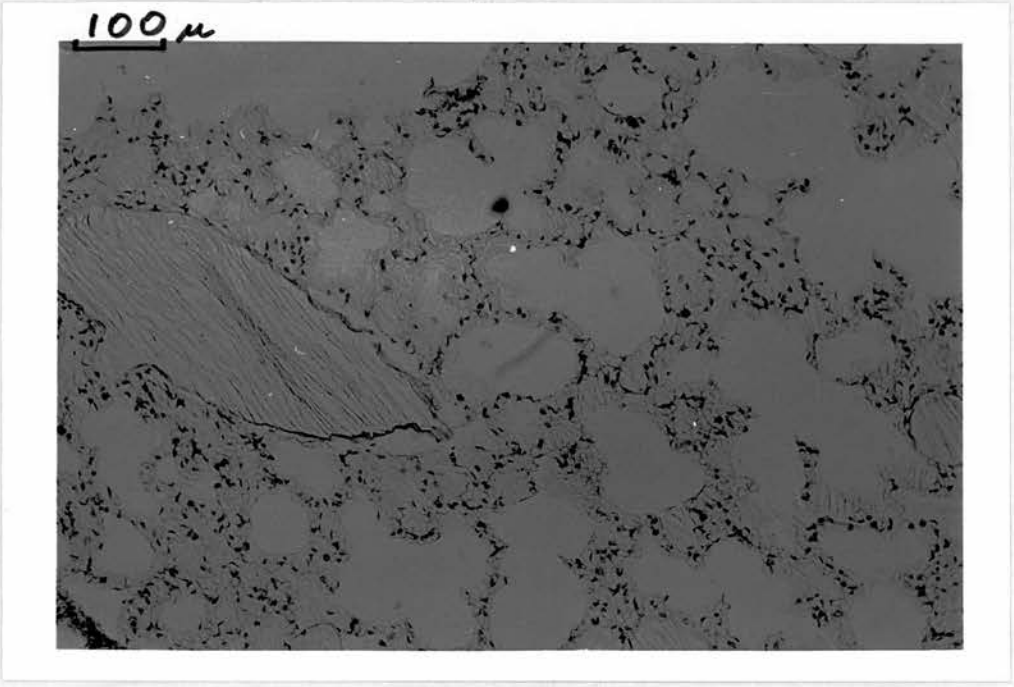


Fig. 42. A pattern of filled and partly-filled alveoli is seen. (Experiment 23).



Figs. 43 and 44. Partly-filled, filled and empty alveoli are seen. (Experiment 23).



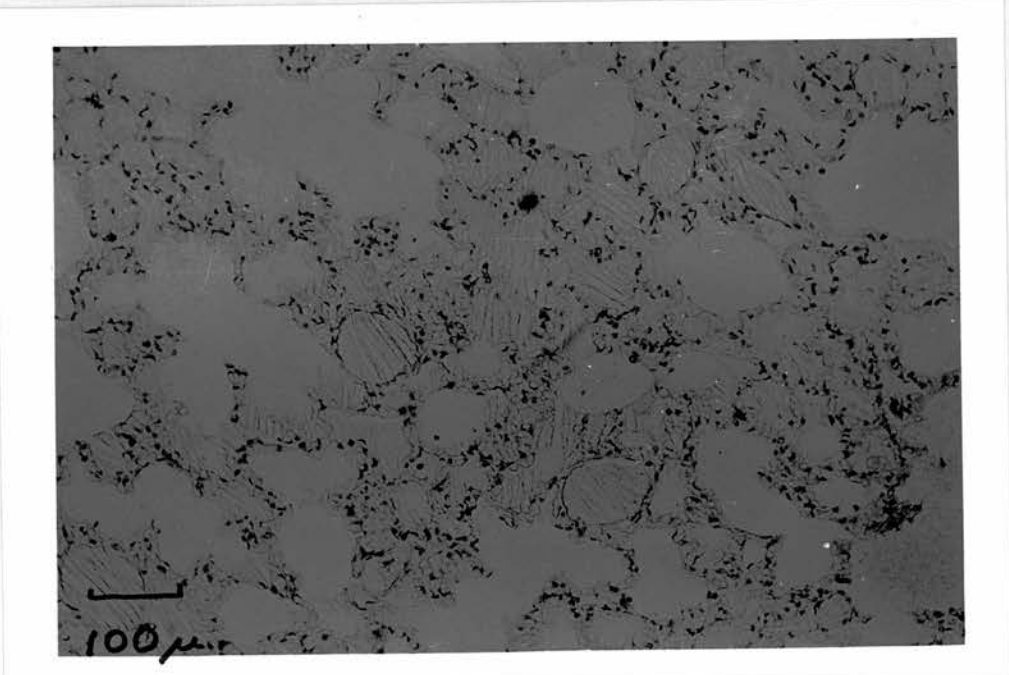


Fig. 45. A flooded alveolar duct (experiment 14).

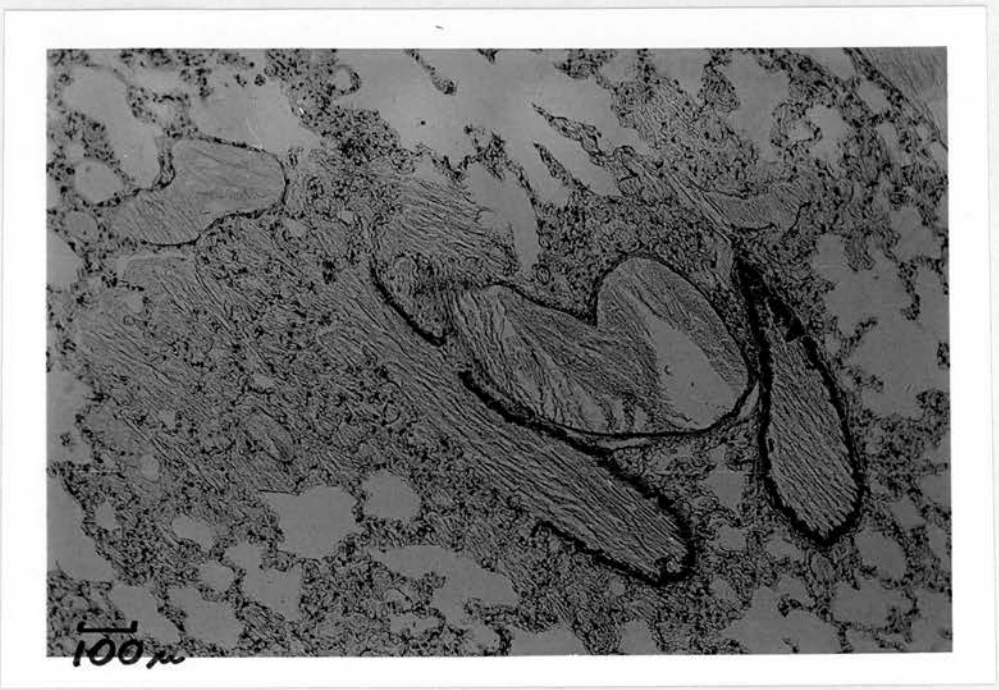


Fig. 46. Experiment 11. Bronchus containing some fluid.

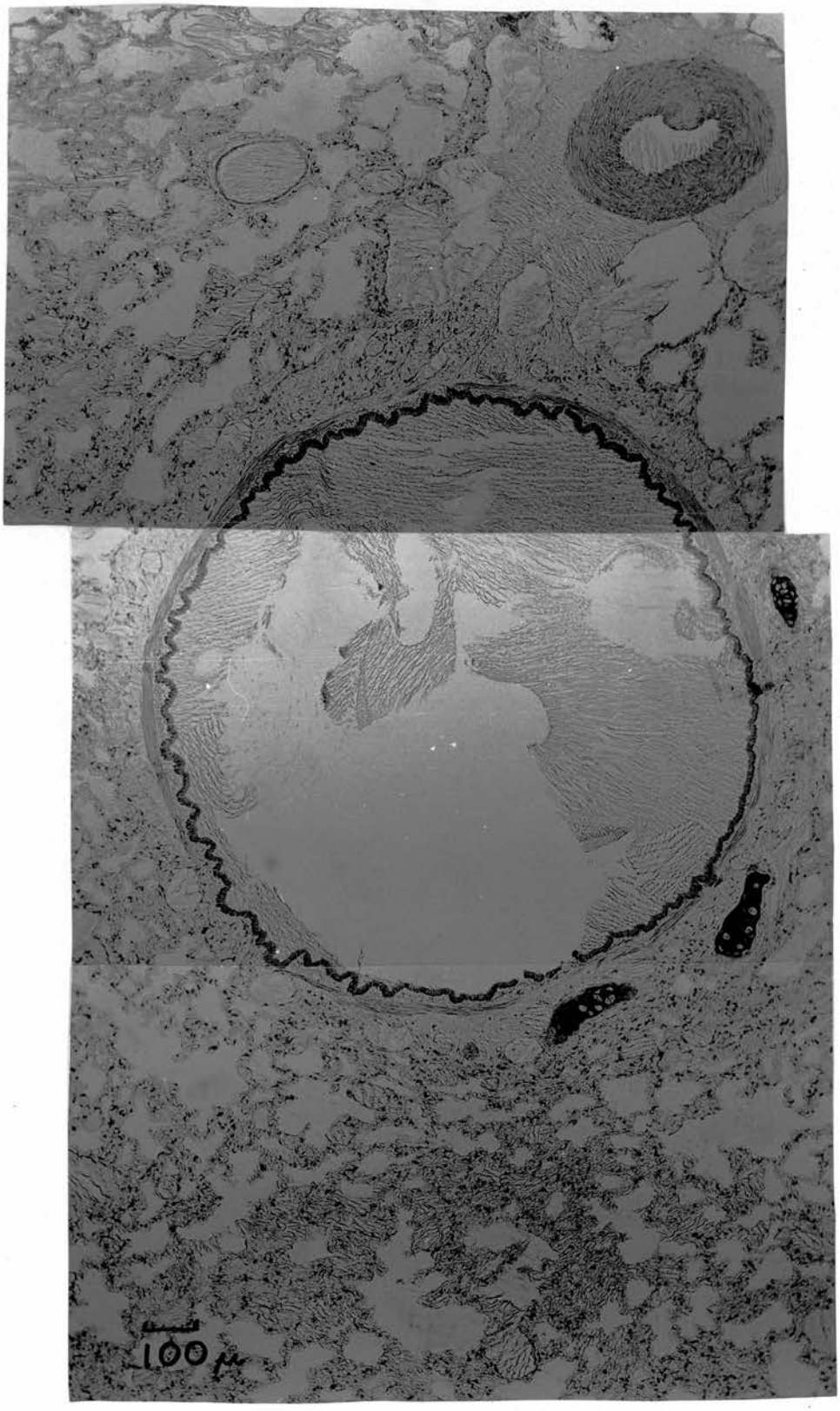


Fig. 47. Areas of alveolar flooding predominantly in the region surrounding large vessels and bronchi (experiment 15).

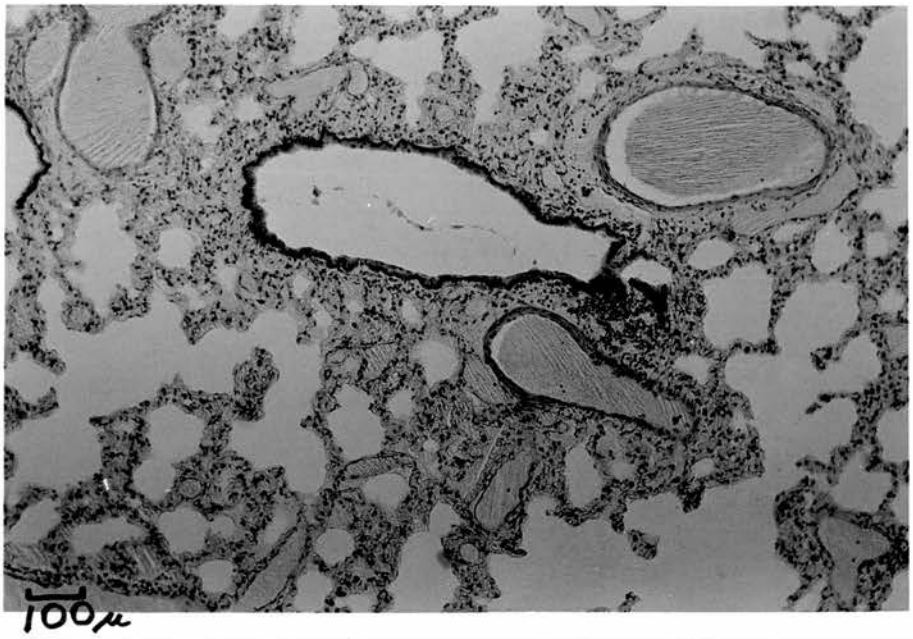
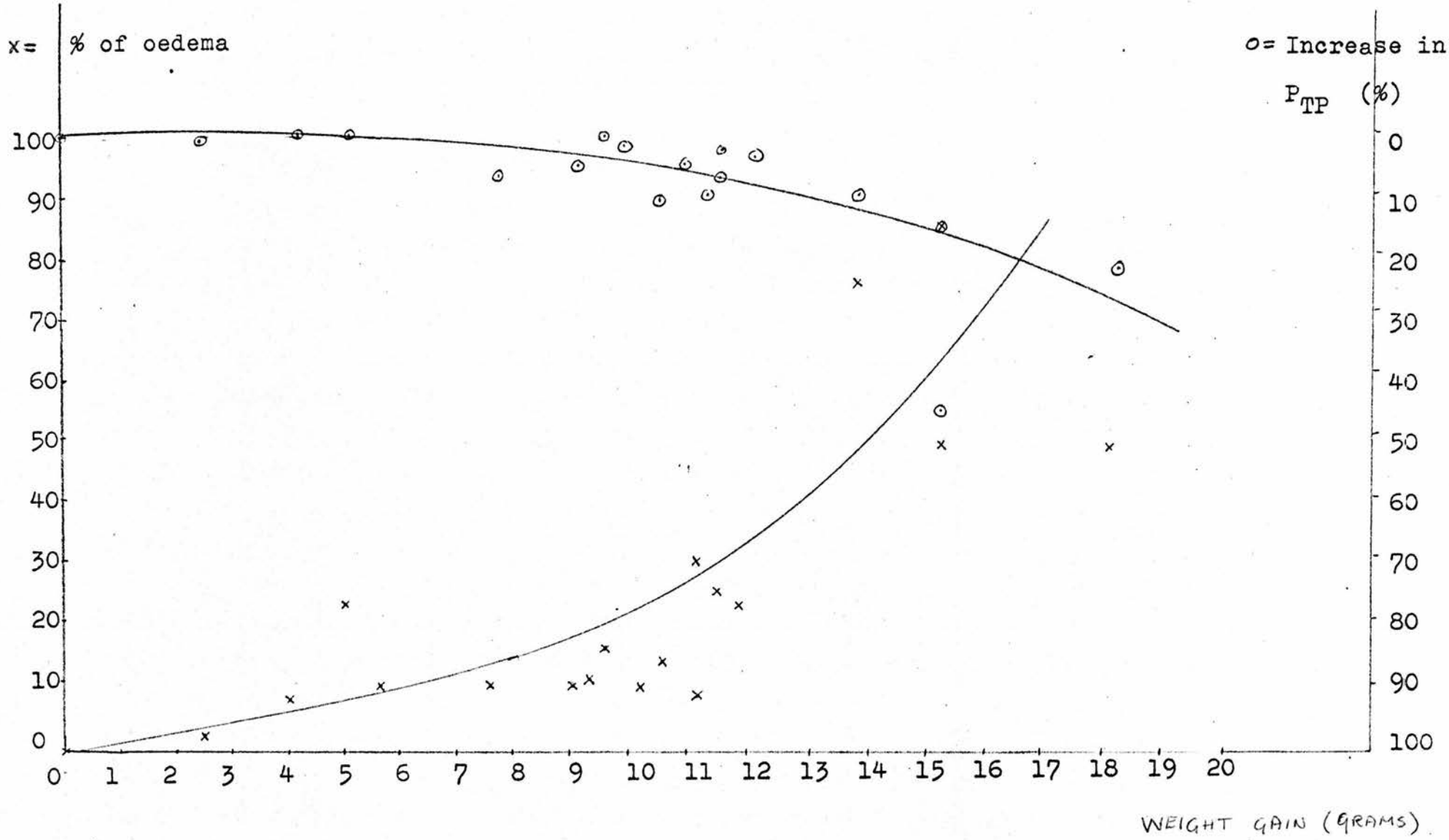


Fig. 48. Percentage of alveolar flooding and weight increase at the point of fixation.



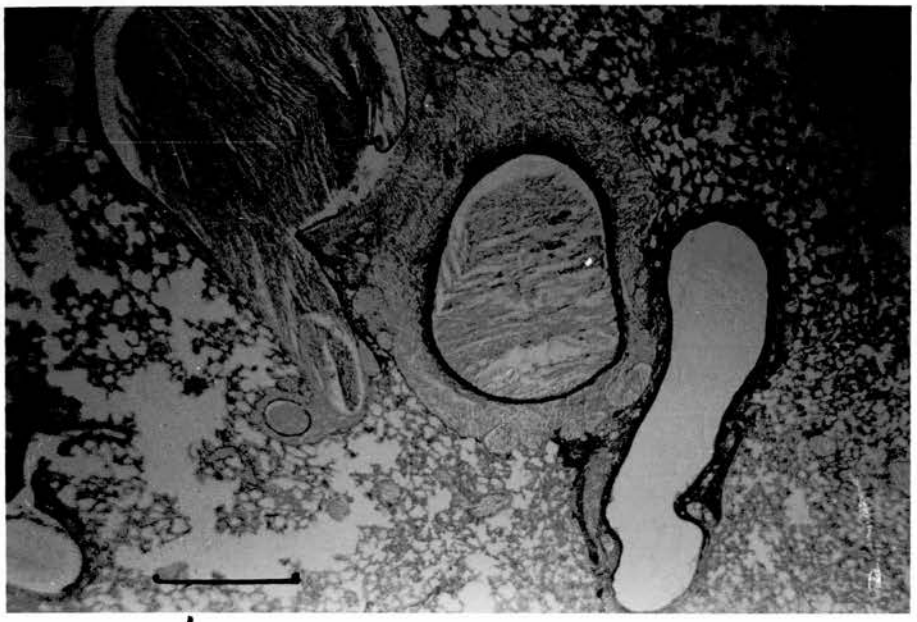


Fig. 49. Perivascular oedema is clearly visible as a cuff around this artery. (Experiment 8).

Fig 50. Perivascular oedema surrounds this artery, and the lymphatics are distended. (Experiment 7).

Fig 51. Oedema fluid is present in a perivascular cuff with filled lymphatic vessels.

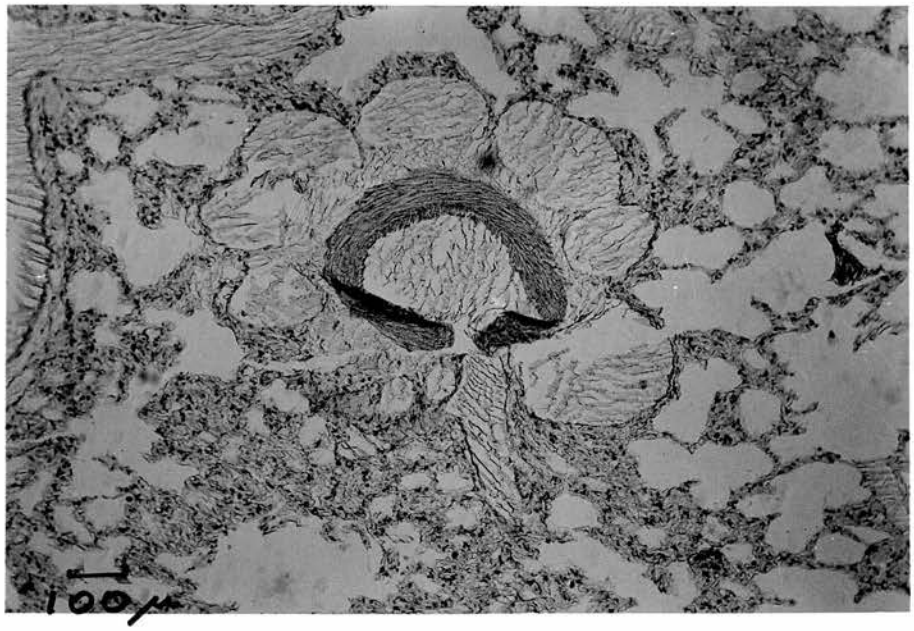


Fig. 52. Experiment 8. Fluid collects predominantly in periarteriolar not beribronchial cuffs.

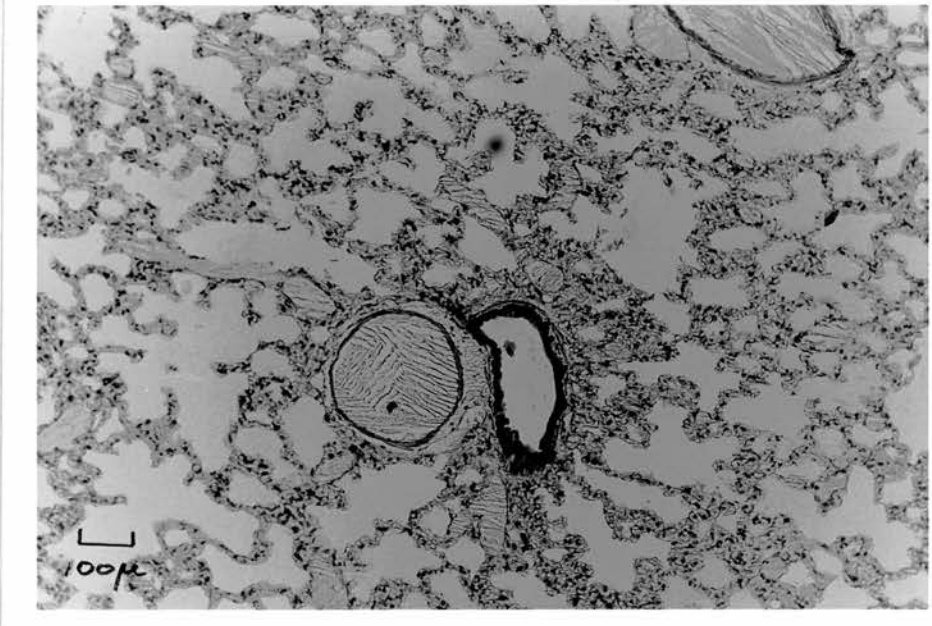
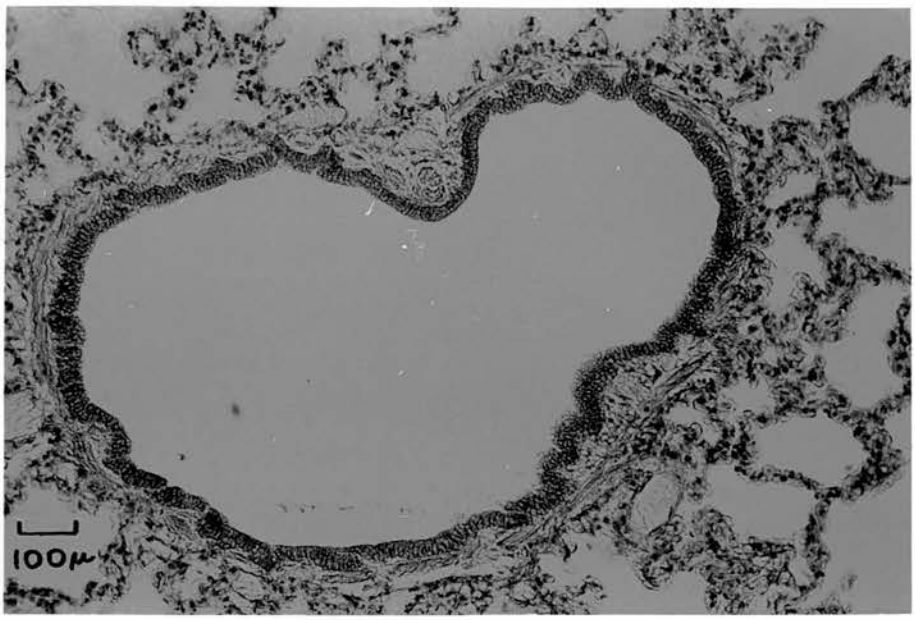


Fig. 53. Periarteriolar oedema is greater in the area between the artery and bronchus. (Experiment 8).

Fig. 54. Little peribronchial oedema is visible (exp.3).



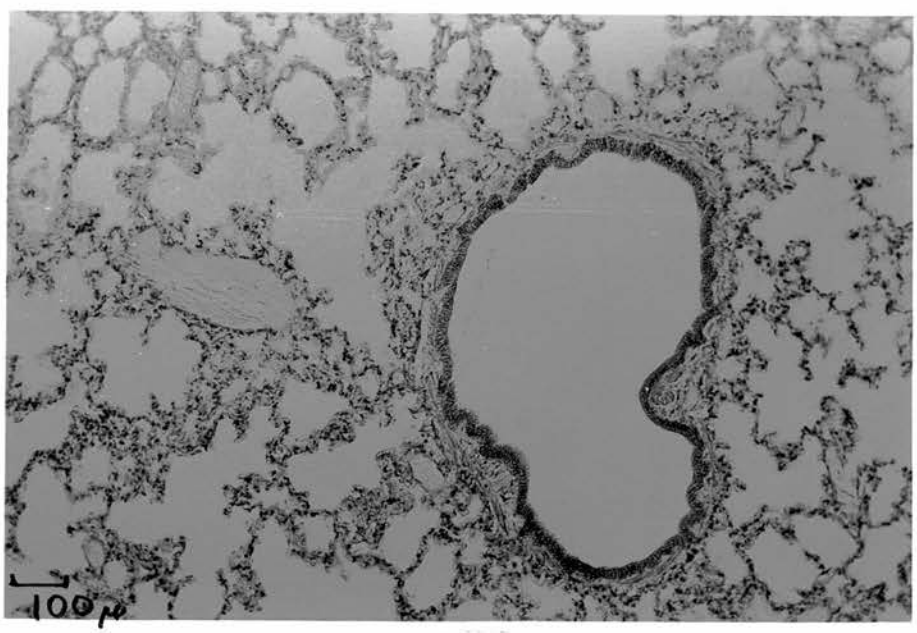


Fig. 55. Only little peribronchial oedema is visible.

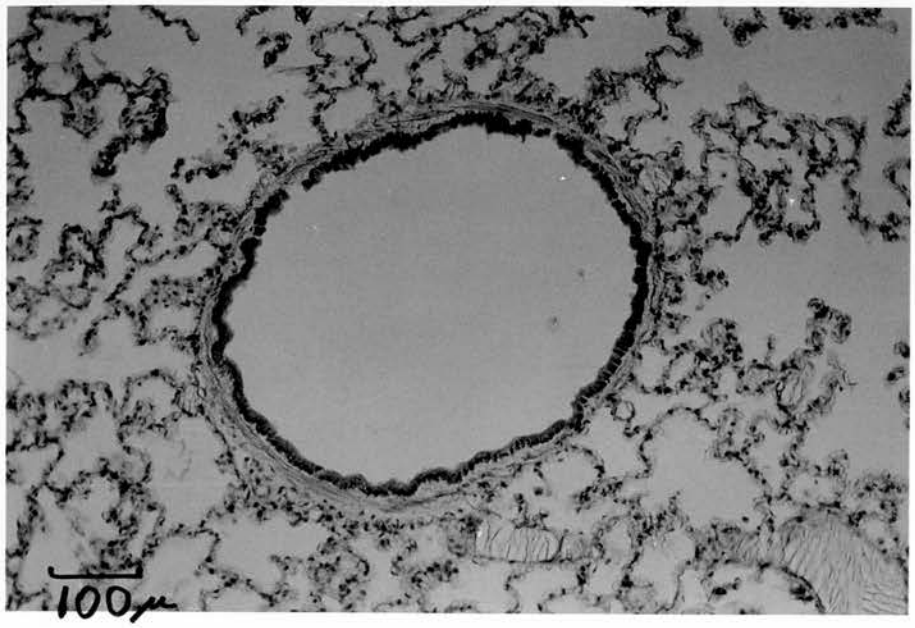


Fig. 56. A bronchial arteriole with a muscular wall and narrow lumen is shown. This is surrounded by considerable perivascular oedema, both within the interstitial space and in distended lymphatics. (Experiment 7.)

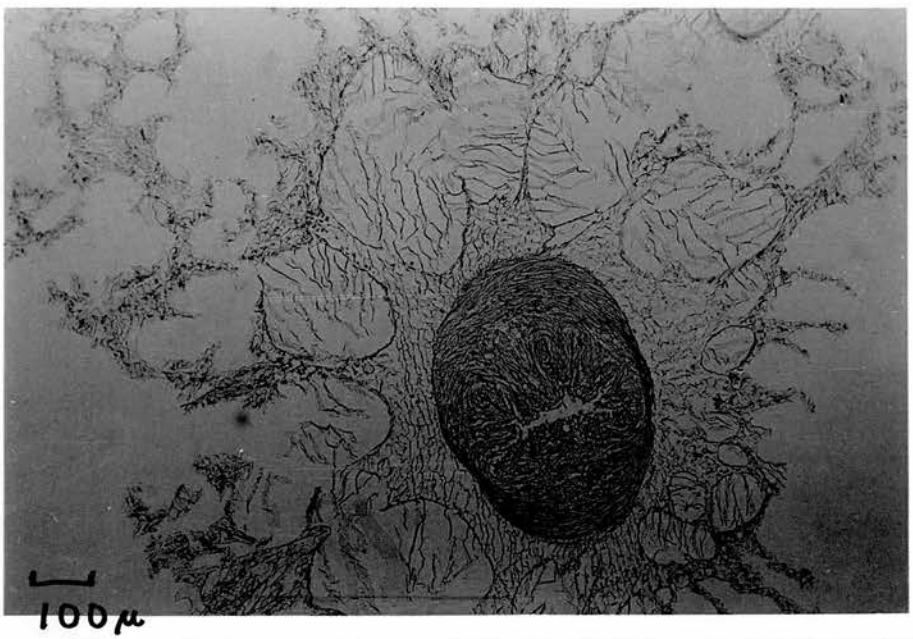
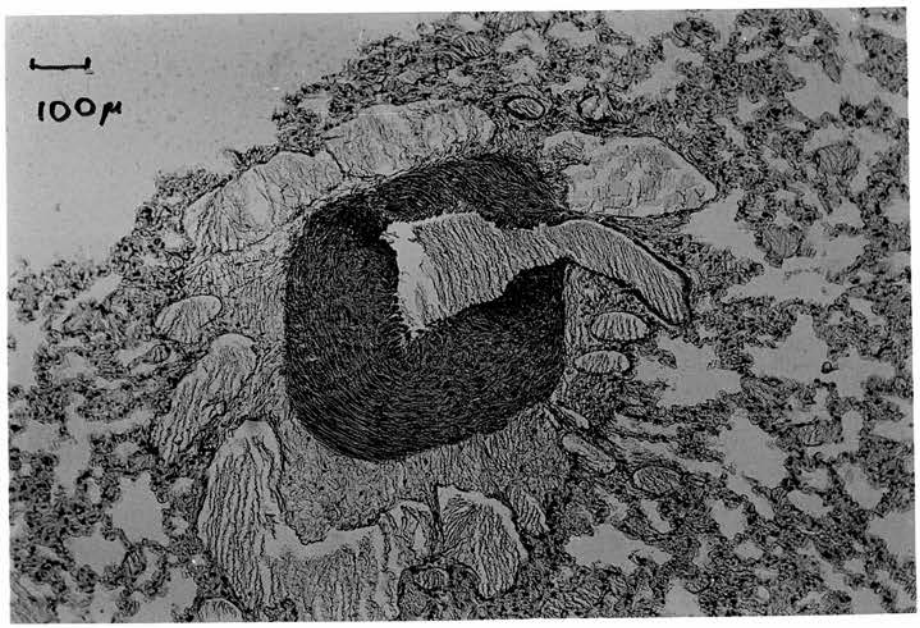


Fig. 57. A bronchial arteriole with thick muscular wall is shown. The perivascular fluid cuff is large and lymphatics are distended.

(Experiment 10)



SECTION 1, PART 1. THE RELEASE OF PGS FROM LUNGS DURING INCREASED HYDROSTATIC PRESSURE AND OEDEMA

DISCUSSION

The Methods Used

(a) The Bioassay System

The aim of the bioassay system was to detect a variety of substances released into the blood or perfusate from lungs *in vivo* and *in vitro*. In particular the occurrence of prostaglandins, their precursors and metabolites was of interest since they were considered likely to be released.

The three main assay tissues RSS, RC and CR were chosen for their characteristic response to prostaglandins E and F, for which they are particularly suitable, although they are also somewhat sensitive to other active substances.

RSS is considerably more sensitive to prostaglandins of the E series than to F prostaglandins, while $PGF_{2\alpha}$ and PGE_2 are equipotent on the rat colon (Gilmore, Vane and Wyllie, 1968; Said, 1974). In the present experiments calibrating doses of $PGF_{2\alpha}$ contracted the RC proportionately more than did PGE_2, but $PGF_{2\alpha}$ was usually much less active than PGE_2 on the RSS (see fig. 9 and 11). The chick rectum was always much more sensitive to the effects of PGE_2 than $PGF_{2\alpha}$, confirming the observations of Said (1974).

Simultaneous contraction of the chick rectum and rat stomach strip demonstrates the presence of PGE-like material, while contraction of the rat colon with little or no contraction of the rat stomach strip would indicate the presence of $PGF_{2\alpha}$ (Piper, Vane and Wyllie, 1970).

That this pattern of response correlates to the presence of PGE and F has been verified by chromatographic, chemical and immunological methods (Gilmore, Vane and Wyllie, 1968; McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee and Ng, 1970; Davis and Horton, 1972; Douglas, Johnson, Marshall, Jaffe and Needleman, 1973). The specificity of the bioassay system is, therefore, the basis for the qualitative identification of prostaglandins although confirmation comes from the disappearance of activity after indomethacin, a specific inhibitor of PG biosynthesis (Ferreira, Moncada and Vane (1971).

The perfused organs were made more specific by pretreatment with the "combined antagonists", (Gilmore, Vane and Wyllie, 1968). This meant that the tissues were blocked against the actions of ACh, 5-HT catecholamines and histamine which might have been released in the intact animal due to systemic hypotension (catecholamines) platelet aggregation (5-HT and ACh, see p.175), or in the isolated lungs (histamine) by dextran.

Indomethacin (5 mg/l) was added to the tissue bathing fluid in most experiments, prior to superfusion to prevent intramural generation of prostaglandins (Eckenfels and Vane, 1972). It did not appear to reduce the sensitivity of the tissues to calibrating doses. In fact the effects of calibrating PGs were often more clearly defined since the spontaneous activity of the tissues was decreased after indomethacin treatment. This was especially important in the rat colon, where spontaneous activity was particularly pronounced, often masking the effects of small calibrating doses.

Vane (1971) showed 100% inhibition of PG synthesis in homogenates of guinea-pig lung with 2 µg/ml indomethacin. The concentration required to inhibit synthesis varies with species and with the pre-

paration tested (Flower, 1974). In the present experiments the assay tissues were pretreated with up to 5 ug/ml which could be expected to inhibit PG synthetase. Doses of up to 12.5 µg/ml in isolated lungs and 3 mg/kg in intact cats were given and could be expected to inhibit synthesis. Only with very much higher doses (20 µg/ml; Northover, 1971) would smooth muscle activity be depressed. This did not occur in the present experiments.

The assay tissues may also detect precursors and metabolites of the prostaglandins, and the rabbit aorta strip was included in several experiments. Rabbit aorta contracting substance (RCS), which is a mixture of TxA_2 with small amounts of PG endoperoxide (Hamberg, Svensson and Samuelsson, 1976) has a half life of 1-2 min in Krebs Ringer solution (Palmer, Piper and Vane, 1973). This is probably longer in plasma, however, since the half like of TxA_2 is about 3 min in plasma but only 30 sec in artificial media (Granström, Kindahl and Samuelsson, 1976; Smith, Ingerman and Silver, 1977). This means that RCS would be detected despite the delay of 1-15 min before the venous effluent reached the assay tissues.

The RbA and RSS are reported to be equally or even more sensitive to the PG endoperoxides and TxA_2 than to PGE_2 and $PGF_{2\alpha}$. Hamberg, Hedqvist, Strandberg, Svensson and Samuelsson (1975) have shown that the RSS is equally sensitive to the PG endoperoxides as to PGE_2 and $PGF_{2\alpha}$ while the RbA is very much more sensitive to the endoperoxides than to E_2 and $F_{2\alpha}$. Bunting, Moncada and Vane (1976), have found PGE_2 to be more potent that the endoperoxides (factor of 2-3) or TxA_2 (factor of 10) on the RSS, and $PGF_{2\alpha}$ more potent than all these substances on RC, whilst on the RbA TxA_2 was more potent than the endoperoxides, and PGs E_2 and $F_{2\alpha}$ were inactive.

Prostaglandin metabolites retain some biological activity (Änggård, 1966). Crutchley and Piper (1975b) have shown that the three pulmonary metabolites formed by the action of PGDH and/or PG reductase were active on the bioassay tissues rat stomach strip, rat colon and chick rectum. The 13-14 dihydro metabolite was 0.2 times as active while 15 - keto PGE_2 and 13-14 - dihydro - 15 keto PGE_2 were only 0.1 and 0.01 times as active as PGE_2.

The bioassay is therefore a good method for detecting ongoing PG synthesis.

The assay tissues were not blocked against bradykinin or angiotensin, which might be expected to be released during platelet aggregation (see Section 2, p. 172) and systemic hypotension (see Section 1, p. 162) respectively.

Calibrating doses of angiotensin II (p. 91) showed a distinct pattern of tissue activity for this substance (figs. 16 and 25). The pattern of assay tissue contraction to bradykinin is shown in fig. 24. The RSS contracts to bradykinin (4 ng/ml), the chick rectum is less active and the rat colon is insensitive. This pattern of contraction differs only slightly from that of PGE_2 and in order to ensure that significant amounts of bradykinin were not released during pulmonary embolism (see Section 2, p.172) the assay tissue, cat jejunum, which contracts specifically to bradykinin, was used.

(b) The Radioimmunoassay

The specificity of the antiserum to $PGF_{2\alpha}$ is discussed in Section 1, part 2. Radioimmunoassay methods and conclusions are discussed on p. 170.

(c) The Intact Cat Preparation

Extensive open chest surgery and an extracorporeal circulation

were required in this preparation. These imposed a considerable strain on the animal causing progressive arterial hypoxemia and systemic hypotension. Although the animals were heparinized, the foreign surfaces of the extracorporeal circulation may have activated platelets causing them to aggregate and become trapped in the pulmonary microvasculature. This caused a tendency to hypotension which was difficult to prevent with transfusions of blood or dextran, and only a limited number of pressure elevations could be performed in each cat.

Infusions of indomethacin caused a fall in systemic blood pressure (fig. 15) which often prevented further pressure elevations being made. The prostaglandins released during pressure elevation may affect the tone of the systemic arteries. Probably the unstable precursors are also important and these are mainly pressor(Rose, Kot, Ramwell, Doykos and O'Neill, 1976). This may explain why the animals experienced grave hypotension when the balloon was inflated after indomethacin treatment, since the systemic pressor effects would be eliminated.

In retrospect, the infusion of macrodex to the animals to maintain blood volume and systemic pressure was inadvisable, since subsequent experiments showed that dextran stimulated PG synthetase in isolated lungs. In fact, only small volumes were infused (up to 50 ml), and in two cats no dextran was infused,and no difference in PG synthesis was observed.

(d) <u>The Isolated Lung Preparation</u>

The isolated lung preparation has been used by many investigators to detect the release of active substances to chemical and mechanical stimuli. Despite the extensive degrading capacity for prostaglandins, they have been reported to occur in the venous outflow of the lungs after a variety of stimuli.

Every attempt was made in these experiments to prepare the tissue with the minimum amount of handling. If the lungs were damaged in any way, if perfusion of the tissue was uneven (i.e. colour was patchy), or if atelectatic areas were observed when the lungs were ventilated, the preparation was discarded. If, as occurred in rare cases, the perfusion pressure increased progressively at the start of perfusion, the preparation was also discarded and a fresh one taken. It was desirable to study PG release during, as far as possible, physiological, not pathological conditions, since only in this way can any findings pertain to the in vivo situation.

The main advantage of using an isolated, instead of intact, lung preparation is that the possible contribution of, for example, release of substances from other organs, or the effects of reflexes can be avoided.

All animals were heparinized fully before removal of the lungs during surgery. This will probably reduce the trapping of platelets in the pulmonary circulation. Also, at the start of perfusion the initial perfusate was discarded after it washed the remaining blood from the tissue; and capillary beds were "recruited" by a short period of elevation of left arterial pressure, which also facilitated washout. Other workers who stun the animals before removing the lungs may have considerable pulmonary trapping of formed blood elements.

Nature of the PGLS and Other Substance(s) Released

Inflation of the balloon catheter in the left atrium of anaesthetized cats caused pulmonary hypotension and congestion, as well as systemic hypotention. This has been described previously in the same experimental model by Hauge, Bö and Aarseth (1977), who showed a

relatively small increase in extravascular lung water in spite of a raised P_{LA} of more than 30 mmHg. Only in a few cases was marked fluid accumulation observed, which they suggested represented alveolar flooding. This corresponds well to the present findings where the P_{TP} appeared to return to normal when P_{LA} was reduced (suggesting re-establishment of the initial intravascular volume and reabsorption of filtered fluid), except in 2 cases where alveolar oedema occurred.

In all 9 cats (Table 3) one or more periods of P_{LA} elevation produced detectable activity on the bioassay tissues, which probably represents PG-like substances, since the tissues were blocked against the action of histamine, 5 HT, ACh and catecholamines (Methods p.46). However, the tissues were not blocked against the effects of angiotension II. The occasionally grave systemic hypotention produced when the balloon was inflated probably stimulated the renin-angiotensin system with subsequent release of angiotensin I. This would then have been converted to angiotensin II in the lungs (p. 8) and released into the arterial blood.

The effects on the tissues themselves of angiotensin II infused in doses up to 1 ng/ml into the superfusate were studied. The RC contracted strongly to angiotensin II while almost no activity was observed on the RSS or CR. However, this pattern of tissue contraction differs substantially from that due to calibrating doses of PGE, and $PGF_{2\alpha}$ (fig. 16) or during periods of P_{LA} elevation (fig. 14). Indeed the contraction of RC during P_{LA} elevation may be completely explained by the effect of angiotensin II. Nevertheless, release of angiotensin II alone is not sufficient to explain the contraction of RSS and CR observed, nor would the release of angiotensin II be inhibited by indomethacin. The return of these tensions to baseline on infusion

of indomethacin in four cats (fig. 14) proves that the substances released were prostaglandins.

Identification of the exact type of PG released is not possible. Tissue contractions matched those of $PGF_{2\alpha}$ most closely, but when the contribution of angiotensin II is taken into account, the substances released may have been PGE_2 plus angiotensin II. However, since prostaglandin intermediates and metabolites are also active on the RSS RC and CR to different extents, and since the contractions of the RbA proved that thromboxanes and possibly endoperoxides were released, it is only possible to say that PG systhesis was stimulated by the pressure increase. The identity of the PG-like compounds released cannot be ascertained.

In 4 out of the 18 pressure elevations no release of PGLS was detected. This might indicate that release is not a constant phenonenon, or rather that the amounts released were too small to be detected. Since the lower level of possible detection in the bioassay was 0.5 - 1 ng/ml PGE_2 and 1-2 ng/ml of $PGF_{2\alpha}$ (figs. 14 and 16), and the amounts released during pressure elevation were of this order of magnitude, a minor reduction in PGLS released would not be detected.

Release of PGs appears to be due to de novo PG synthesis, as little or no PGs are stored in the tissues (Piper and Vane, 1971). Although angiotensin II in several tissues and cells is reported to stimulate PG synthesis and release (McGiff et al. (1970), Gimbrone and Alexander (1975), see p. 173) infusion of angiotensin II to cause increased levels in blood entering the lungs did not release PGLS into arterial blood (fig. 15). The increase in the blood level of angiotensin II was higher than the maximal increase during hypovolemia (0.33 ng/ml; Hodge, Lowe and Vane (1966), Hall and Hodge, 1971); but no release of PGLS was observed since the tissue patterns to infused

angiotensin II before and after indomethacin treatment were identical.

Stimulus to Release PGLS

The mechanical stress due to increased vascular hydrostatic pressure may well stimulate PG synthesis. Piper and Vane (1971) have indirect evidence that leakage of fluid into the extravascular space, rather than stretching of vessel walls, releases prostaglandins: isolated perfused guinea-pig lungs sensitised to ovalbumin released PGs only when an irreversible increase in weight of the lungs occurred. In lungs where pulmonary venous pressure is increased there is an initial rise in pulmonary blood volume due to distension of vessels and recruitment of new capillary beds (Section 3, p.181). There follows a net outward filtration of protein-containing fluid from the exchange vessels into the interstitial space, considerably distending it. Lymphatic drainage increases, and, if filtration continues, fluid enters the alveolar spaces of the lung (Staub, Nagano and Pearce, 1967).

It is possible then that PGs could be released from the lungs in pulmonary oedema, perhaps as the tissue extracellular space is filled with, and expanded by, fluid filtering from the exchange vessels. In fact, Said and Yoshida (1974) report that pulmonary oedema released PGs. Apparently fluid extravasation may be a stimulus for PG release. However, fluid accumulation in the interstitium is usually rather scarce when P_{LA} is elevated in intact cats (Hauge, Bø and Aarseth, 1977) and in one of two cases where fulminant alveolar oedema developed no PGLS were released. Also in the isolated lung experiments where we induced a slow filtration of fluid into the extravascular space by

means of a moderate rise (10-15 mmHg) in left atrial pressure in plasma perfused cat and rabbit lungs (Table 1, Group A and fig. 6) there was clearly no release of prostaglandins, nor were PGLS detectable in tracheal fluid bioassay (fig.7). This would indicate that release of PGs in intact cats was a result of the very considerable elevation of hydrostatic pressure and not due to fluid filtration from the exchange vessels.

In isolated perfused lungs (Table 1, Group B) outflow pressure was raised to 20, 25 and in one case 30 mmHg, with no observed release of PGLS, although pressure elevations of similar magnitude in intact cats did cause release (figs. 8 and 9). This would imply either:
(a) that release in isolated lungs was below the limit of detection of bioassay (see p.26) or
(b) that the lungs are not responsible for the observed release, or
(c) that inflating the balloon activated some mechanism in the intact cat which released PGs from the lungs. This mechanism must not have existed in the isolated lung.

Another possible explanation for the discrepancy between the results from isolated perfused lungs and intact animals may have been due to the species difference, since initial experiments were performed on isolated rabbit lungs. This could also have explained the discrepancy observed between these results in isolated lungs where no release was observed, and those of Said's group where PG release was detected in cat lungs, and this was a strong argument for the importance of species variation in causing the apparent anomalies. In fact even differences in strain within a species have been shown to cause marked differences in biological responses to pharmacological stimuli. Accordingly, two isolated cat lungs

were tested (Table 1) under identical perfusion conditions as for rabbit lung preparations, with horse plasma as perfusate. In one pair of cat lungs slow filtration of fluid was not accompanied by detectable PG release (Table 1, Group A, experiment 7) while in a second pair of cat lungs (Table 1, Group B, experiment 12) perfused with horse plasma induction of hydrostatic pulmonary oedema was rapid (P_{LA}=20 mmHg), causing considerable sudden increase in blood volume and distension of vessels. These lungs also failed to release prostaglandins.

The importance of perfusate characteristics and the role of platelets in PG release was then considered. Platelets are found in large numbers in the pulmonary microvasculature (Kaufman, Airo, Pollack and Crosby, 1965) and may be trapped there in microaggregates, or adhere to the capillary walls, and might have been the source of at least part, of not all, of the PGLS detected from the intact cats. Platelets are capable of releasing large amounts of prostaglandins during aggregation (Smith and Willis, 1970; Hamberg and Samuelsson, 1973), and their importance in pulmonary embolism is discussed in Section 2 (p. 172). The extracorporeal circulation may aggravate the formation of microemboli when blood comes in contact with foreign surfaces and these emboli will be trapped in the vast pulmonary microvasculature and may release PGLS. Increasing hydrostatic pressure may then 'wash out' PGLS from previously occluded capillaries, which would then be detected on the assay tissues. This is unlikely for two reasons. The release of PGLS was maintained during periods of P_{LA} elevation of up to 10 minutes duration(in all but one case), an unlikely occurrence if the prostaglandins were preformed and simply entering the systemic arterial circulation. Also the effect of indomethacin and the activity on the RbA must strongly

suggest de novo systhesis. The origin of these newly synthesized PGs may have been the platelets. However, in two cat lungs perfused with heparinized cat whole blood no PG release was detected during grave vascular distension and subsequent pulmonary oedema (Table 1, Group B, experiments 13 and 14). Therefore the lack of platelets in artificial perfusates is not the factor responsible for lack of PG release in the isolated lungs, and it is extremely unlikely that PGLS released in intact animals originated from the platelets.

Recently attention has been focused on the role of PGs as local hormones or intracellular messengers rather than circulating hormones (Silver and Smith, 1975) and this is consistent, in the case of PGs of the E and F series, with their short life in vivo (see p.15). Mathé and Levine (1973), using radioimmunoassay, reported release in anaphylaxis of the pulmonary metabolites of prostaglandins, the 15 - keto 13, 14-dihydro - prostaglandins, with little or no release of parent prostaglandins. Also Liebig, Bernauer and Peskar (1974) have detected high amounts of the 15-keto 13, 14-dihydro metabolite of $PGF_{2\alpha}$ amongst the other active substances released during anaphylaxis. One explanation is that an active PG degradation mechanism downstream from the site of PG release may metabolize part or all of the prostaglandins, which have been synthesized and have acted as local hormones within the tissue or vessel walls, before they leave the lung via the pulmonary circulation. If this is indeed the case it is of importance to try to detect not only parent prostaglandins but their pulmonary metabolites also.

The site of pulmonary degregation of PGs is the capillary endothelial cells (Ryan, Niemeyer and Ryan, 1975). Although no PG release into arterial blood was detected, there may have been stimulation of synthesis in the tissue itself, which would be detectable as an

increased tissue content of PG or as high levels in the tracheal fluid. In both these instances PGs would probably have escaped degradation.

However, the concentration of $PGF_{2\alpha}$ in homogenized lung tissue was not significantly different in oedematous than in control lungs. (Table 6a). Also RIA measurement of $PGF_{2\alpha}$ in tracheal froth detected only small amounts of this substance (1 - 5.56 ng/ml) which did not differ from that detected in perfusate samples (0.7-3.58 ng/ml), and was considerably lower than the concentration of PG (20 ng/ml $PGF_{2\alpha}$) reported by Chijimatsu, Hara and Said (1976). In the single experiment where 15-keto 13, 14-dihydro $PGF_{2\alpha}$ in the perfusate was measured, the concentration of this metabolite more than doubled during 45 minutes of perfusion, increasing from 1.35 to 3.3 ng/ml (fig. 18). In the same experiment there was a corresponding decrease in the plasma concentration of the parent prostaglandin, $PGF_{2\alpha}$. It must be noted that the lungs were perfused in a recirculating system. Possibly the high initial plasma level of $PGF_{2\alpha}$ was due to the effect of removal and perfusion of the organ on stimulation of PG synthesis, and subsequent breakdown of this to 15-keto 13, 14-dihydro $PGF_{2\alpha}$ in the circulating plasma would be sufficient to account for this substance's increase.

These experiments with radioimmunoassay, which is capable of detecting much lower concentrations of PG than is possible by bioassay, show that no increased synthesis of PGF_{α} occurred during hydrostatic pulmonary oedema.

Localization and Cause of PG Synthesis

It was important to ascertain whether the PGLS released from intact cats were from the lungs or not. It is known, for example, that PGs are released into blood during haemorrhagic shock (Jakschik,

Marshall, Kourik and Needleman, 1974). If PGLS are released from extrapulmonary tissues they would have to traverse the lungs to appear in arterial blood. Since the catabolism and inactivation of PGs in the pulmonary circulation is extremely efficient (70-90% ; Ferreira and Vane, 1967a; Piper, Vane and Wyllie, 1970 see p. 94) it would require very high levels of PGs in the mixed venous blood reaching the lungs to produce activity equivalent to 1ng/ml in carotid arterial blood.

It might be argued that the increased PG-like activity in arterial blood of intact cats is due to decreased pulmonary inactivation of PGs. Whether increased vascular hydrostatic pressure will influence PG degradation in endothelial cells is unknown. However, some factors might actually tend to increase PG inactivation : increased vascular surface area due to distension in the microcirculation, as well as reduced cardiac output will increase the exposure time of the pulmonary endothelium to blood. In fact fairly extensive damage to the pulmonary endothelium has to occur before inactivation is impaired (Jose et al, 1976) during pulmonary hypertension in man. Jakschik et al (1974), who report peak arterial concentrations of 2.6ng/ml of PGE_2-like material in carotid arterial blood of dogs after severe haemorrhagic shock, have suggested either that the pulmonary degradation of PGs must be impaired or that the lungs are the source of these PGs, perhaps as an autoregulatory readjustment of blood flow as occurs in the kidney (Herbaczynska-Cedro and Vane, 1973), and the heart (Alexander, Kent, Pisano, Keiser and Cooper, 1973). To ascertain whether pulmonary degradation of PG was altered during pressure elevation, exogenous PGE_2 was infused at normal pressures. A value for pulmonary degradation of about 80% was found and this was unaffected by elevation of P_{LA} (fig. 17 ; and Table 4).

On the other hand the assay tissues are somewhat sensitive to the pulmonary metabolites of PGs (Crutchley and Piper, 1976) although sensitivity is only 1/10 to 1/100 of that of the parent PGs. Consequently percentage pulmonary degredation may have been even higher than 80%, since the effects of the metabolites on the tissues might give a falsely high estimate of the amount of unchanged PGE_2.

Whatever the actual value for the pulmonary degredation of prostaglandins it remains the same during alterations in vascular pressure. It is therefore very likely that the PGs were released from the lungs themselves, probably distal to the site of destruction.

The systemic hypotension caused when pulmonary hydrostatic pressure was raised (Table 3) may activate the sympathetic nerves to the lungs and cause PG release (see p. 19). Increased sympathetic activity is known to cause PGE synthesis in several other organs (Hedqvist, 1976) and may have been the stimulus for release here, since stimulation of sympathic nerves to the lungs has been reported to release PGs (Mathé et al, 1977).

The Effect of Perfusate Characteristics on Stimulation of Prostaglandin Synthesis in Isolated Lungs and Its Significance

When studying which stimuli release PGLS from isolated lungs most investigators have used Krebs Ringer solution (Palmer, Piper and Vane, 1973; Lindsey and Wyllie, 1970), or Krebs Ringer dextran (Said and Yoshida, 1974) as perfusate. Also, in cases where this is reported, the flow rates used were very low, for example 8 ml/min in rat lungs (Alabaster and Bakhle, 1970; 1976) or 5 ml/min in guinea-pig lungs (Mathé and Levine, 1973). This may facilitate the detection of relatively low quantities of prostaglandin, although it may be disadvantageous

in that it could be insufficient to maintain the viability of the lung preparation and cell damage may occur. The principal reason for using such a low flow rate might be to delay the development of oedema, and this could also account for the fact that most investigators do not ventilate the lungs, although another reason for not ventilating guinea-pig lungs is their high airway resistance (Table 1, Group B, experiments 15,16 and 17). Egan (1976) has shown that the solute permeability of the alveolar epithelium is a dynamic function of the inflation of the lungs. That is, the calculated equivalent pore radius varied from 0.5 nm at low levels of inflation to large leaks at high levels. When isolated lungs are perfused over a long period of time, pulmonary oedema develops spontaneously, the actual length of time varying between lungs and depending on the type of perfusate used (Nicolaysen, 1971a). Release of vasoactive substances into the perfusate may contribute to this change in capillary permeability.

In the experiments of groups A-E (Table 2) the effects of various perfusates in oedema formation due to extended perfusion were investigated in twelve isolated, ventilated rabbit lungs. In two lungs perfused with Krebs Ringer solution, oedema development was fairly rapid (15 min after the start of perfusion). Despite perfusion of these lungs for up to 3-5 hr (Table 2, Group B) no tissue contractions were observed. Apparently PGs are not released in the general pathological decline in viability of the lungs during extended perfusion. This suggests that PGs probably do not contribute to the non-specific damage causing oedema in isolated perfused lungs although more direct studies, for example with synthesis inhibitors, would be needed to prove this. Similarly, in horse plasma perfused lungs (Table 2, Group B) where plasma colloid osmotic pressure was approximately normal there was no release of PGs, although lungs were

perfused until gross oedema was observed (fig. 11).

Krebs Ringer Dextran solution has been used as a perfusate in isolated organs since the osmotic pressure closely approximates that of plasma, and oedema formation is delayed. In three KRD perfused lungs a spontaneous release of PGE-like substance was observed 100-150 min after the start of perfusion (fig. 10). In one lung little fluid filtration had occurred (3-6 g). Thus the release of PGs must have been due to dextran, since a) oedema does not release PGs during prolonged perfusion in plasma or Krebs Ringer perfused lungs, and b) if oedema were the stimulus for PG release in KRD perfused lungs, it would be difficult to account for release in lungs where very little fluid filtration had taken place (3-6 g filtered in lung 1, Table 2).

Moreover, no PG release was seen in plasma or Krebs Ringer perfused lungs during development of hydrostatic pulmonary oedema (Table 1 Groups A and B) while PG synthesis was stimulated during increased P_{LA} in KRD perfused lungs (Table 1, lungs 9, 10 and 19).

The addition of indomethacin to the perfusate (50-125 ng/ml) abolished the gradual rise in tissue baselines, which occurred in the KRD perfused lungs approximately 90-150 min after the start of perfusion, confirming that tissue activity was due to PG release (figs. 10 and 13). However, plasma perfused lungs did not release PGLS and indomethacin added to the perfusate (figs 7 and 8) had no effect on tissue tensions.

One very interesting finding in dextran perfused lungs was the release of small amounts of PGLS (0.5 - 1 ng/ml), about 90 min after the start of perfusion, to short lasting stimuli such as hyperinflation, short periods of raised outflow pressure and of raised flow to the lungs (figs. 10 and 13). None of these stimuli produced equivalent

responses on the assay tissues when they were performed in plasma or KR perfused lungs (fig. 11), which would suggest that dextran has a facilitating effect on PG synthesis in lungs. Interestingly Hyman, Bennet, Joiner, Chapnick, Mathé and Kadowitz (1976), and Hyman, Mathé, Spannake and Kadowitz (1976) have reported enhanced PG synthetase activity in dextran-perfused dog lungs.

Dextran has been reported to release histamine from mast cells 3-5 min after exposure (Rowley and Benditt, 1956) and a similar dextran-induced release of histamine occurs from lung tissue strips (Gryglewski, Dembiska - Kiec, Grodzinska and Panczenko, 1976). No reports have been published which show the time taken for dextran to release prostaglandins, but the responses seen here to hyperinflation and raised vascular pressure were immediate (figs. 10 and 13) when the delay time for the perfusate to reach the assay tissues is taken into account. Nevertheless, at least 90 min lapsed from the start of perfusion before the gradual release of PGLS was seen and it was only then that raised pressure or hyperinflations stimulated release. Similar stimuli at the start of perfusion had no effect. It is tempting to speculate that dextran must cross from the pulmonary exchange vessels into the interstitial space before it can affect PG synthesis. Accordingly, in the three pairs of KRD perfused lungs where outflow pressure was raised and net outward fluid filtration enhanced, the time taken to release PGs was less than in those lungs where perfusion at normal vascular pressure was extended. Since the mean molecular weight of Dextran T 70 is 70,000, its equilibration with interstitial proteins may be slow.

Blocking either H_1 receptors alone, or in combination with H_2 receptor block (but not H_2 receptor block alone) abolishes PG release

(fig 12). Release still occurs in lungs perfused with KRD containing H_2 receptor blocker (fig. 13). The simplest explanation for this would be that dextran causes histamine release with subsequent stimulation of the H_1 receptor which then leads to the synthesis and release of PGs. Dextran might release both compounds from mast cells, but the finding that PGs were not detectable during H_1 receptor blockade is then difficult to explain. It would appear that PG release is secondary to the release of histamine and its action on H_1 receptors. Substantiating this are reports that histamine infusions cause PG synthesis in isolated lungs (Gryglewski et al, 1976; Leibig, Bernauer and Peskar, 1974) and in lungs of intact animals (Yen, Mathé and Dugan, 1976).

Mathé, Volicer and Puri (1974) reported that previous H_1 receptor blockade diminished the output of $PGF_{2\alpha}$, while H_2 blocker pretreatment decreased the liberation of PGE_2 but not $PGF_{2\alpha}$ from lungs. In experiments using bioassay it is , of course, not possible to identify conclusively either of these PGs. Also stimulation of PG synthesis may lead to the release of a variety of intermediates and chemically related compounds, for example endoperoxides and thromboxanes, and these would also be active on the tissues (p.148). The PG pattern detected, however, resembled PGE_2 calibrations more closely than $PGF_{2\alpha}$ although all release could be prevented by H_1 receptor blockade.

If the PG released during perfusion with dextran is predominantly PGE_2, which is a vaso- and broncho-dilator in most species (Bergström, Duner, von Euler, Pernow and Sjövall, 1959; Bergström, Carlson, Ekelund and Öro, 1965; Carlson, Ekelund and Öro, 1969; Nakano, 1973), it will have the effect of lowering systemic blood pressure and may

contribute to the hypotension observed when an anaphylactoid reaction occurs during infusions of dextran in man (Lorenz et al, 1976). It may also have a beneficial effect of improving the microcirculation and tissue perfusion during infusions of dextran.

The Possible Significance of the PG Release in Intact Animals

Infusion of arachidonic acid increases pulmonary vascular resistance in dog lungs in vivo, probably through conversion to the endoperoxides, $PGF_{2\alpha}$ and PGE_2. $PGF_{2\alpha}$ is a more potent vasoconstrictor than AA (1,000 times more potent; Hyman, Bennet, Joiner et al, 1976). Pulmonary bronchial infusion of $PGF_{2\alpha}$ has been shown to increase lung vascular resistance and decrease compliance (Gold, Lapierre, Levison, Bryan and Orange, 1976). PGE_2 increases pulmonary arterial pressure and vascular resistance in intact dog, swine and lamb lungs (Kadowitz, Joiner and Hyman, 1975), while in isolated lungs PGE_1 causes vasodilatation (Hauge, Lunde and Waaler, 1967).

Intermediates in the biosynthesis of prostaglandins possess much greater biological activity than the natural prostaglandins. The cyclic endoperoxides have a short half life; when endoperoxides, or their stable analogues, are injected intravenously they are highly potent pulmonary vaso- and broncho-constrictors (Kadowitz, Chapnick, Joiner, Matthews and Hyman, 1976; Wasserman, 1975). In fact, the endoperoxides are probably the biologically active form of the prostaglandins in the lung, and PGE_2 and PGF_{2x} may be the less active metabolites. Indeed thromboxane A_2, an important product of PG conversion in the lungs (Hamberg and Samuelsson, 1974; Hamberg, Svensson and Samuelsson, 1976), is an even more potent broncho-constrictor than PGH_2 (Svensson, Strandberg, Tuvemo and Hamberg, 1977).

Prostaglandins also have potent effects on airways, which may be very important if PGs are released from lung tissue for example during sympathetic stimulation. Injections of $PGF_{2\alpha}$ into pulmonary lobar arteries increase airway resistance by constricting airway smooth muscle (Kadowitz, Joiner and Hyman, 1974). Hirose and Said (1971) have injected PGE_1, $F_{2\alpha}$ and A_1 into the jugular vein of anaethetized dogs. $PGF_{2\alpha}$ caused vasoconstriction while PGE_1 and A_1 were vasodilators. The PGs were also active on the airways, $PGF_{2\alpha}$ causing peripheral and central airway constriction. Similarly, Drazen and Austen (1974) have measured an increase in respiratory resistance and a decrease in compliance after i.v. infusion of $PGF_{2\alpha}$, histamine and bradykinin in the unanaesthetized guinea-pig.

The sensitivity of the pulmonary vessels and airways to the prostaglandins and the huge synthetic capacity of the lungs would suggest that they play some regulatory role, perhaps in maintenance of the ventilation perfusion ratio.

Prostaglandins and their intermediates also have potent cardiovascular effects (Emerson, Jelks, Daugherty and Hodgman, 1971; Rose et al, 1976). Thus the function of the systemic and pulmonary circulations and airway tonus may be directly altered by release of PGLS and, indirectly, through modulation of function in the autonomic nervous system.

Another possible consequence of the release of prostaglandins from lungs during increased hydrostatic pressure and pulmonary congestion is their possible effect on the kidneys. PGE has been shown to be a potent renal vasodilator and stimulator of natriuresis (McGiff and Itskovitz, 1973; Johnston, Herzog and Lauler, 1967; Martinez-Maldonado, Tsaparas, Eknoyan and Suki, 1972; Strandhoy, Ott, Schneider,

Willis,Beck, Davis and Knox, 1974) by its direct effect on the kidney. (Vander, 1968.) This would suggest a possible physiological role for PGE released from the lungs acting intra-renally to alter the sodium and water balance and alleviating pulmonary congestion.

Conclusion

In intact cats during increased pressure in the pulmonary circulation PG-like activity, measured in arterial blood by bioassay, is elevated as long as the pressure is increased, or until indomethacin is given. This indicates prostaglandin release, probably from the lungs since PG-degrading capacity is not altered although extremely high concentrations released systemically might have been detected. However, release does not occur in isolated lungs of cat, rabbit and guinea-pig, when perfused with plasma, whole blood or Krebs Ringer solution. Neither stretching of the vessels nor fluid filtration cause release into the perfusate or the tracheal fluid of isolated lungs. Release is dependent on the intact state of the animal and is probably due to reflex sympathetic stimulation of the lungs when vessels are stretched, or when systemic hypotension occurs. The ability of dextran in the perfusate of isolated lungs to stimulate PG synthetase was shown and this could be prevented by histamine (H_1) receptor blocker. PG release into arterial blood will influence smooth muscle tone in both the pulmonary and systemic circulations.

SECTION 1, PART 2. DISCUSSION AND VALIDITY OF RADIOIMMUNOASSAY OF PGF_{α}

RIA has been used extensively to measure PGF_{α} and PGE *in vivo*, especially in human plasma and serum (Caldwell, Burstein, Brock and Speroff, 1971; Jubiz, Frailey, Child and Bartholomew, 1972; Gutierrez-Cernosek, Morill and Levine, 1972) and the values published vary considerably. (200 pg/ml to 1.5 ng/ml for PGF_{α}).

Samples for radioimmunoassay of $PGF_{2\alpha}$ were extracted as described and dissolved in Tris buffer. Several samples were purified on a silicic acid column and the fraction containing PGF_{α} was collected. These samples showed values of PGF_{α} approximately 20% lower than those obtained in samples not extracted on the column.

The specificity of the assay (i.e. of the antiserum to PGF_{α}) was tested by assaying known concentrations of PG metabolites. The results are shown in fig. 20 as the amount of unlabled compound needed to displace 50% of $^{3}H\text{-}PGF_{2\alpha}$ from rabbit $PGF_{2\alpha}$ antiserum. The antiserum is equally specific to both $PGF_{1\alpha}$ and $PGF_{2\alpha}$ hence the use of the general term, PGF_{α}, while it is 100x less specific for 15 keto 13-14 dihydro $PGF_{2\alpha}$. All other PGs and metabolites have much loweraffinity for the antiserum.

The high values reported here are not entirely due to the cross-reaction of the antiserum with other non-specified compounds, since this accounts for at most 20% of the observed activity. However, values as low as 2 pg/ml have been reported by Samuelsson, (1973) when calculated from production rates measured by combined gas chromatography and mass spectrometry. When urinary metabolites are measured after i.v. administration of labled PGE and PGF, the true concentration

of these PGs may be less than 20 pg/ml (Samuelsson, 1973). Also other studies report values of 12-84 ng/ml in normal human plasma (Jaffe, Berhman and Parker, 1973; Dray, Charbonnel and Maclouf 1975). This latter group, in a careful methodological study, have emphasized the importance of chromatographic separation before assay. Also a large volume of plasma was used (10 ml) and care was taken to remove all blood cells, especially platelets which are a rich source of prostaglandins. A combination of these factors may account for the high values observed in the experiments reported here. However, the values measured here may be correct, and the content of PG in perfusate of isolated organs may well be higher than that in plasma of intact animals.

Since isolated, perfused lungs are capable of degrading over 95% of exogenous $PGF_{2\alpha}$ (Piper, Vane and Wyllie, 1970), the detection of up to 3.4 ng/ml in the perfusate during perfusion suggests that there is a continuous output of $PGF_{2\alpha}$ from these lungs. Substantiating this is the increase from 1.36 to 3.28 ng/ml in the concentration of the 15-keto 13-14 dihydro metabolite of $PGF_{2\alpha}$ during 44 minutes of perfusion, while the concentration of $PGF_{2\alpha}$ fell over the same period of time (fig. 18).

Nijkamp, Moncada, Whittle and Vane (1977) have reported a continuous basal release of arachidonate from sensitized Krebs Ringer-perfused guinea-pig lungs, and Ferreira, Moncada and Vane (1971; 1973) have suggested that there is a basal release of PGs from isolated kidney. Similarly Kadowitz, Chapnick, Joiner and Hyman (1975) showed PG release in isolated, perfused dog lungs. This may be due to cell damage, or, more likely, to the effect of handling the tissues on PG synthesis.

SECTION 2. PULMONARY MICROEMBOLISM AND PG RELEASE

DISCUSSION

Type of Smooth Muscle Active Substances Released

In the present experiments, whenever a collagen infusion caused transient increases in peak inspiratory pressure the assay tissues RbA, RSS, RC and CR contracted. This would indicate a release of PGLS (Piper and Vane, 1971) but the bioassay method does not enable positive identification of any specific PG. It is unlikely that the PGLS released were purely PGE_2 or PGF_{2x} since standard doses could not duplicate the responses after collagen infusion, and since contractions of RbA were observed: this tissue is sensitive to TxA_2 and to the PG endoperoxides (p.150). Probably a variety of products in the conversion of arachidonic acid were released.

PG synthesis is blocked by indomethacin (Vane, 1969) and in 2 cats, assay tissue activity was abolished after i.v. doses of indomethacin, confirming that PG release had occurred. However, indomethacin has a dual action in this situation. The aggregation of platelets is also prevented when PG synthesis is inhibited (Hamberg and Samuelsson, 1974), since mediators produced from arachidonic acid are involved in collagen-induced platelet aggregation. The formation of emboli will therefore be prevented.

Other non-PG smooth muscle active substances may have contributed to the tissue activity observed. Tissues were not blocked against bradykinin, and collagen is known to activate Hageman factor (Niewiarowski, Bankowski and Regowiska, 1965), which in turn may activate the kallikrein-kinin system (Cochrane, Revak, Wuepper, Johnson,

Morrison and Ulevitch, 1974). The results obtained with the CJ as an assay tissue demonstrated, however, that measurable amounts of kinins did not reach the bioassay tissues (fig. 24) although kinins might still have been generated during collagen infusions: bradykinin; with a half like of less than 20 sec in blood (Ferreira and Vane, 1976b) could have been inactivated before reaching the assay tissues and thus have escaped detection.

The transient hypotention observed after collagen infusion might have activated the renin-angiotension system causing release of angiotensin II. This could explain most of the contraction of the RC, and thus the difference between the patterns of contractions, during ex vivo and in vivo platelet aggregation (fig. 23). Therefore, although angiotensin II and possibly bradykinin may have contributed slightly to the tissue activity detected, the major part of the activity was due to PGLS.

Site of Prostaglandin Systhesis

The present experiments allow no definite conclusion as regards the origin of the PGLS released. Platelets are known to synthesize and release PGs during their aggregation (Smith and Willis, 1970; Willis et al, 1974). When platelets were aggregated 'ex vivo' by infusing collagen into the extracorporeal circulation PGLS were released from the platelets, giving a pattern of tissue activity not very different from that during in vivo aggregation. Apparently PG release from platelets is sufficient to account for the observed tissue responses without having to postulate any release from the lung itself. However, the concentration of collagen infused over the tissues was four times greater than that given in vivo to produce the same contractions. Some PG release

from the lung tissue itself, possibly stimulated by the presence of microemboli lodged in small vessels, cannot be excluded.

On the other hand, when platelet aggregates are trapped in the pulmonary microcirculation their release of PGs probably takes place close to reactive smooth muscle cells. In the immediate vicinity of the aggregated platelets and pulmonary smooth muscle cells, the concentration of PGs is probably higher than in mixed blood from the lungs. The PG-like activity in arterial blood might also be reduced by pulmonary inactivation of the PGs released (Ferreira and Vane, 1967a), and the activity detected in the tissues after i.v. doses may have been mainly due to metabolites. A direct comparison of the two collagen doses can therefore not be made.

Evidence from non-biological embolisation of isolated lungs perfused with blood free medium shows that PG release from lung tissue occurs at least occasionally (Palmer, Piper and Vane, 1973). However, pulmonary microembolism in platelet free animals caused only weak pulmonary responses (Bø, Hognestad and Vaage, 1974).

It would seem that platelets are the likely source of PGs in the experiments reported here, possibly with some additional PG release from the lungs.

The Relationship Between PG Release, Platelet Aggregation and Lung Responses

Rådegran, et al (1977) have shown by radioimmunoassay techniques that $PGF_{2\alpha}$ is released during prothrombin, and thrombin-induced platelet aggregation in dogs. PG activity in blood is increased during intravascular platelet aggregation (Rådegran, 1971; Vaage and Piper, 1975) and PGs have been suggested as possible mediators of the lung responses.

In the present experiments PGLS were detected only whenever lung responses were elicited. No PG release occurred when lung responses and platelet aggregation were "exhausted" by repeated collagen infusions. Undoubtedly a relationship exists between the release of PGLS and the lung responses to platelet aggregation. Whether the PGLS caused the lung response has not been ascertained. Several factors suggest that PGLS, in the amounts detected, could have caused the pulmonary broncho- and vaso-constriction. Some of the PGLS (for example $PGF_{2\alpha}$, thePG endoperoxides and TxA_2) formed during the conversion of AA are smooth muscle constrictors in the lung (Kadowitz, Joiner and Hyman, 1975; Kadowitz, Chapnick, Joiner and Hyman, 1976) and there is pulmonary vasoconstriction during infusion of AA into isolated lungs (Wicks, Rose, Johnson, Ramwell and Kot, 1976). Although in the present experiments only low concentrations of PGs were detected in blood leaving the lungs, concentrations in the immediate vicinity of the trapped platelet aggregates may have been much higher, and capable of causing constriction of the surrounding vascular and bronchial smooth muscle; and some may also have been degraded before reaching the mixed pulmonary venous blood. It is reasonable to suggest a causal relationship between the PGLS and the lung responses observed, although there is no direct evidence for this from these data.

During the platelet aggregation and release reaction other active substances are discharged which may contribute to smooth muscle constriction (Kobayashi, Mashino, Herther and Didishein, 1974). Among the substances released from platelets are adenine nucleotides, serotonin and histamine. Experiments using pharmacological blockers have shown that serotonin and histamine contribute to the pulmonary effects of induced intravascular platelet aggregation (Rådegran, 1971;

Swedenborg, 1971; Hageman, Wentling and Pruss, 1973).

Tucker, Weir, Reeves and Grover (1976) suggested that prostaglandins are the primary if not the sole mediators of the airway responses they observed to microembolism. They have shown that the increased pulmonary vascular resistance caused by particulate microembolism is attenuated but not abolished by combined PG and histamine blockade, while the alveolar hypoventilation observed was mediated by prostaglandins alone.

There is some disagreement as to whether ADP and ATP are vasoconstrictor or vasodilator (Hauge, Lunde and Waaler, 1966, Swedenborg, 1971; Vaage, Bø and Hognestad, 1974). It is unlikely, therefore, that adenine nucleotides, serotonin and histamine could be the principle mediators of the lung effects reported here, although they may have contributed to them.

Conclusion

Intravascular platelet aggregation due to collagen infusion in intact cats is accompanied by PG synthesis and release whenever platelet aggregation and pulmonary vaso- and broncho-constriction occur. It is likely that the various substances formed during conversion of AA to PGs produced the assay tissue responses observed. Release of these substances, either from platelets, lung tissue, or both, is probably sufficient to explain the pulmonary smooth muscle constriction, although the involvement of additional vasoactive substances cannot be excluded.

SECTION 3. HYDROSTATIC PULMONARY OEDEMA AND CHANGES IN LUNG COMPLIANCE

DISCUSSION

Validation of the methods used:

1. Fixation Procedures

Both the fixation procedures used produced good results for light microscopy. However, for the present purpose, the epon-embedding method was less useful than the paraffin-embedding method of the rapidly-frozen lungs, in that the former gave blocks of a very much smaller size for sectioning and many blocks had to be sampled. The osmium penetration into the tissue and the cutting of epon limits block size (Hayat, 1970). On the other hand, fig. 27 (cross section of a block from the lower lobe of one experiment) shows the area which is seen in a paraffin section. Usually 2 or more blocks were made from each experiment, and thence sliced to ensure that large areas were viewed and sampling was representative.

Gravity has a profound effect on the pulmonary circulation (West, 1970). West has proposed a division of the lungs into 3 functional zones dependent on the effective circulatory pressures. At the apex of the upright lung ventilation exceeds perfusion since the hydrostatic pressure is inadequate to force the blood so high (Zone 1). At the base of the lungs perfusion exceeds ventilation (Zone 3). Between these zones, ventilation and perfusion are relatively well matched (Zone 2). The major part of the normal lung lies in this zone. However,

elevating P_{LA} means that more of the lungs are under Zone 3 conditions. This uneven distribution of oedema will affect the distribution of blood flow and ventilation, causing arterial hypoxemia under clinical conditions.

Lungs showed a marked vertical gradient of pulmonary oedema. This was noted both for interstitial oedema, since the pulmonary interstitium is a continuum and for alveolar oedema, which was greatest in the dependent part of the lungs. Because of this, care was taken to examine sections from both middle and lower lobes in all experiments. In addition, in glutaraldehyde perfused-fixed lungs sections from the upper lobes were also examined, although this was not possible in rapidly frozen lungs.

2. Light Microscopy and the Point Counting Methods

i) Light Microscopy (LM):

Both perfusion- and rapid freezing-fixed lungs showed good preservation of tissue structure for LM, although this was very much better in perfusion-fixed lungs. In fact, samples from lungs fixed by glutaraldehyde perfusion also showed preservation of normal lung structure on electron microscopic examination (unreported observations). Although this was not so for freeze-fixed lungs, in the present experiments they had a greater advantage: the freezing procedure is rapid, and mechanical changes in the living lung are stopped in 0-10 sec (Staub and Storey, 1962). This is important when studying oedema, since delays in the prefusion fixation method, perhaps coupled with changes in the intravascular osmotic pressure,

might alter the fluid balance and create artifacts.

With rapid freezing, true tissue dimensions are preserved to at least 0.4 cm below the pleural surface (Kulka, 1961). Although much deeper areas were examined in the experiments reported here, this was considered valid since no actual measurments were made. Only the ratios of filled to non-filled alveoli and of interstitial cuff to vessel or bronchial lumen were of interest. In fact, since the area of interest for the quantification of interstitial oedema was the loose connective tissue round the extra-alveolar conducting vessels and the airways, it was necessary to examine areas remote from the pleura.

One important observation made by Staub, Nagano and Pearce (1967) is that alveolar flooding is an all or nothing phenomenon - that is, no partly-filled alveoli are seen. I did not confirm this observation. In fact, partial flooding was a fairly frequent occurrence, with a concave meniscus seen at the entrance to the alveolus (figs 39, 43 and 44). Also gas trapping was seen (fig 40). I suggest this was due to the positive pressure method of ventilation used here, which may hold open alveolar sacs.

No conclusions can be drawn as to where the fluid filtration which caused the alveolar flooding took place. It may be as Iliff(1971) has claimed, that not only does it occur in the so-called exchange vessels (capillaries) but also in large extra-alveolar vessels.

ii) The Point-Counting Method:

a) experiments fixed by vascular perfusion

In this preliminary series of experiments an attempt was made to quantify alveolar oedema by counting the number of filled and non-filled alveoli observed in each field viewed under the light microscope. The number of filled alveoli was expressed as a percentage of the total number of alveoli counted (Table 7a). A little alveolar flooding was observed in experiments where no secondary fall in C_L had occurred. Since this might have been caused by the fixation method itself, or the results may have been biased by insufficient sampling the freeze fixation method was developed for use in the next set of experiments.

b) experiments fixed by rapid freezing

Any method using counts and/or measurements of structures in histological sections has errors in it, some of which can be predicted and to a certain extent corrected for (Weibel, 1963). The simplified method of Caldini, Leith and Brennan (1975), adapted to this project, involved calculating only the ratio of two structures. It was therefore considered unnecessary to correct for section thickness. It also obviated the necessity of calculating the tissue volume fraction, if a structure were to be quantified. However, it is recognized that filled alveoli occupy a smaller volume than do non-filled ones (Staub, Nagano and Pearce, 1967; also see figs 39-44). The ratio of filled to non-filled alveoli, and thus the percentage of alveolar flooding (Table 7b and fig 48) would be slightly underestimated, although this error would be small and was not considered to affect the conclusions of the study. The results from the point counting method in these experiments agree fairly well with those calculated for perfused fixed lungs, although the number of alveoli counted in the latter experiments was much smaller.

Pulmonary oedema and changes in lung compliance

The results of these experiments confirm those of Hauge, Bō and Waaler that raising P_{LA} caused an initial steep fall in C_L. The reported effect of raising P_{LA} is to increase intravascular volume (Lunde and Waaler, 1969) without increasing extravascular water as measured by the indicator dilution technique (English, Digerness, Kirklin and Karp, 1971). This lowered C_L. Possibly, as Frank (1959) has shown, this is due to a competition for space between blood and gas, reducing functional residual capacity or the volume of terminal airways. The competition may be at the site of alveolar capillaries and venules.

When P_{LA} was raised in my experiments, there was almost a doubling of preparation weight. The increase was certainly due in the main to increases in intravascular volume of the preparation. In preparations where P_{LA} had been raised, capillary lumens were visibly distended compared to controls. While some interstitial fluid was observed in all preparations where capillary filtration had occurred, especially in distended lymphatics, no interstitial distension could have accounted for the large increase in volume. Also, in some preliminary experiments it was noted that returning the elevated P_{LA} to zero at any time before the secondary decrease in C_L resulted in a rapid rise (within 10 sec) of C_L to control levels. This suggests decreased intravascular volume and not resorbtion of interstitial fluid caused the return of C_L, since interstitial fluid resorbtion is a slow process.

The results of quantifying interstitial oedema (Table 8) are hard to explain. There is no correlation between increases

in lung weight and the amount of interstitial oedema. The most probable reason for this is that insufficient areas were viewed and counted. Qualitatively, however, the light microscope pictures showed that periarterial and peribronchial interstitial compartments were capable of large distension.

The secondary fall in C_L, shown as an increase in P_{TP} in fig 48, coincided in this preparation with an increased weight of 9-12g. The fall began gradually, then accelerated. An increase in P_{TP} of 10% of the P_{TP} value measured immediately after the rise in P_{LA} was accepted as proof of this fall having taken place. Two experiments, 21 and 22, showed a clear increase in P_{TP} (20 and 45%) and marked alveolar oedema. Of more interest were the results from lungs fixed at a weight gain of 8-12g. All showed about 10% of alveoli were filled. These were lungs fixed at the start of the secondary decrease in C_L.
Perhaps one surprising finding was that in lungs fixed after only small weight gains (4-5g in experiments 4,5 and 10) there was alveolar oedema. This is not easy to explain. It does not necessarily mean that the sequence of fluid accumulation in oedema outlined by Staub, Nagano and Pearce (1967) did not happen here for one or more of the following reasons:

i. not enough points were counted. This is unlikely since in one experiment (21) where there was considerable alveolar flooding it was calculated that the counting of about 2000 points (overlying alveoli) was enough to give a reliable result, since the result did not change when more points (up to 6000) were counted.

ii. the sampling method in these lungs could have been

biased towards the dependent areas. This is probably true. In fact, it was impossible to sample from the upper lobes which were damaged by the clamping and freezing procedure. Also this distorted the lung shape and it was often difficult to locate precisely the area I wanted to sample. A bias towards dependent areas in rapidly frozen fixed lungs may account for the observation that flooding, although present , was less in perfusion-fixed lungs at comparable weight increases, where it was easier to define sampling areas.

iii. the alveolar oedema may be an artifact. For example, atelectatic areas could have been incorrectly scored as "filled alveoli". Also, since lungs were fixed in end expiration, alveolar volume was small, and it was occasionally difficult to distinguish between filled and non-filled alveoli. It is unlikely that this caused too large an error since many sections were counted and, when uncertainty arose, the area or point was simply not scored. However, some errors were probably made.

iv. since isolated lungs have no intact lymphatic drainage, and this is an important mechanism of oedema prevention, this may have affected the oedema formation in these preparations. Nevertheless, an interesting point to note is that huge fluid-filled lymphatic vessels were seen in many sections in the perivascular interstitium (figs 49-51,56 and 57), which shows that lymphatics were at least capable of being filled, and perhaps of passively draining fluid filtered from the isolated lungs.

v. damage to these particular lungs may have caused alveolar flooding. A single step elevation in this preparation to 15

or 20 mmHg may be too high. It could have caused too severe a distension of the vascular bed and focal haemorrhages could have occurred in the dependent areas.

A combination of these factors may account for the alveolar flooding in experiments 4,5 and 10, since no single factor can be completely responsible. It is probably true to say that a small amount of alveolar flooding took place before the secondary decrease in C_L was measurable., although the values in Table 7b may be an overestimate.

Relevance to the Clinical Situation

This was an experimental method of inducing acute pulmonary oedema in normal lungs. It can not be compared with the more chronic pulmonary congestion which is encountered clinically, especially during prolonged mechanical ventilation, and which may alter the mechanical behaviour of the lungs. Also, during acute respiratory failure (or the wet lung syndrome) there are diffuse changes in the pulmonary vasculature causing increased capillary permeability and the loss of plasma into the interstitial space. This is certainly not analagous to the experimental situation reported here and may be more easily reproduced by agents which alter vascular permeability.

The time course of my experiments was fairly short, lasting at most 2 hours since controlled induction of hydrostatic oedema was the aim. Moreover, as has been discussed in section 1 part1, during prolonged perfusion isolated lungs undergo changes from which oedema develops spontaneously. This may explain the apparently conflicting results of Reith et al (1972) , where the experimental timecourse was longer and where, after relief

of venous congestion in isolated dog lungs a further one hour of perfusion took place before microscopic examination of the preparation was made, at which time there was clear evidence of interstitial oedema

Conclusions

A primary fall in C_L (increase in P_{TP}) was observed and correlated with increased intravascular volume in isolated lungs, although some interstitial oedema was observed in all lungs where P_{LA} had been raised. Weight gains of up to 10-12g produced considerable interstitial oedema and small amounts of alveolar flooding, while C_L remained constant or fell very gradually. At larger weight gains (15-18g) lungs showed marked alveolar flooding and a clear decrease in C_L. However, the start of the secondary decrease in C_L (at an increase in P_{TP} of 10-15%) was not clearly defined. It could not be exactly correlated with the start of alveolar flooding, as the latter was seen in certain preparations before the decrease in C_L was observed. Nevertheless, it did correlate with a greatly accelerated rate of alveolar oedema development.

Further work

It would be of great interest to discover the exact nature of the PG-like substances released during the elevation of P_{LA} in the intact animal experiments. More specific methods than the bioassay used here would be necessary. For example, development of the radioimmunoassay method outlined in Section 1.2. Two very important questions could then be answered. Firstly, the exact cause of the PG release when P_{LA} is raised could be identified (e.g. reflex sympathetic stimulation due to decreased systemic blood pressure?). Ganglion blocking drugs would be useful tools here. Also the effects of the release on the lungs themselves and on the systemic circulation, especially the kidney, could be examined using, for example, techniques to measure natriuresis. Are the PGs, in the amounts released, sufficient to cause the postulated effects on the kidneys?

Another important point arising from this work would be to examine further the mechanisms by which dextran releases PGs and the involvement of the histamine receptors.

In section 2 the role of PGs as mediators of vascular and airway constrictor responses to microembolism was not proved, since indomethacin prevents platelet aggregation itself. Possibly similar experiments with ADP-induced platelet aggregation could subatantiate these results. Also selective inhibition of TxA_2 (by 5HIAA) would be interesting. However, the exact nature of the released PGs should be examined, for example by RIA.

The experiments in Section 3 raise very many interesting questions. The most important one is the nature of the decay of the preparation during prolonged perfusion, which occurs even when the perfusate is iso-osmotic with plasma. Although this has already been extensively examined, it has not been answered, and relates to the important area of organ preservation for transplants.

APPENDIX

Perfusates.

Krebs Ringer solution (KR).

Composition (mM) : NaCl 118.65, KCl 4.64, CaCl 2.51, KH_2PO_4 1.183, $MgSO_4$ 1.184, $NaHCO_3$ 24.64 and glucose 5g/l.

Horse plasma was obtained by centrifuging heparinized (30IU/ml) whole blood at 1000xg for 10 min. Plasma was filtered before use.

Cat whole blood was obtained by cardiac puncture of anaesthetized cats which had been given heparin (500IU/kg)i.v. 1000 units of heparin was added to each 100 ml blood.

Krebs Ringer Dextran solution (KRD) was made by dissolving 27 g/l of Dextran T70 (mol. wt. 70,000) in Krebs Ringer solution. The osmolarity varied between 260 and 285 mOsm.

Measurement of pH. The pH of the perfusate, or of cat blood,was measured at intervals throughout each experiment (Acid-Base Analyzer PHM71, Radiometer, Copenhagen, Denmark).

Measurement of Osmolarity. Osmolarity of the perfusates used was measured before and at intervals during the experiments using an Advanced Digimatic Osmometer, Advanced Instruments Inc., Needham Heights, Mass., USA. The osmolarity of plasma in intact cats remained between 320-328 mOsm/kg throughout each experiment.

Measurement of Hematocrit. Hematocrit measurements in intact cats gave values of 0.27-0.35 at the start of the experiments. Throughout the experiments a progressive reduction to values ranging from 0.15-0.28 was observed.

Force Transducer. A Sanborn FTA-100-1 force transducer was used for the measurement of changes in weight of the isolated lung preparation. A counterbalance of 50g was suspended and connected to the transducer by means of a catgut thread over a minimum friction two-wheeled pulley.

Pressure Transducer. P_{PA}, P_{FA} and P_{LA} were recorded with Statham P23D6 pressure transducers. P_{TP} was measured by a differential pressure transducer.

Recorders. Weight changes were amplified and recorded on a 2-channel Hewlett Packard Oscillograph recorder (model 700 2A). Full scale deflection was 5g. Pulmonary tracheal pressure was also recorded, full scale deflection usually 10 cmH_2O. P_{PA}, P_{FA} and P_{LA} and assay tissue tensions were recorded on an 8-channel Grass Polygraph model 7B.

Recording of bioassay tissue contractions. Tissue contractions were recorded isometrically by transducers made in the mechanical workshop of the Preclinical Medical Institute of the University of Oslo, Norway. Transducer design is shown in figs. 3 and 4. The element was protected against light by a metal box. With full scale sensitivity not less than 0.4g no instability, drifting (in 4 hr.) or non-linearity of the transducers was observed. The semiconductor transducer elements (AE 801-803) were obtained from A/S Microelectronikk, 3191 Horten, Norway.

Blood Donors. Cats weighing 1.5-2.5 kg. were used as blood donors in intact cat bioassay experiments. They were anaesthetized with 30-40 mg/kg Nembutal® (Sodium Pentobarbitone) i.p. and given 500 I.U./kg heparin "Novo" i.v. Blood was withdrawn by cardiac puncture and 1000 I.U. heparin/ 100 ml blood was added.

Drug Infusions. Drugs were infused to the intact cats by a constant volume infusion pump, model 747, Harvard Apparatus Co., Mikis, Mass., USA.

Monitoring of blood flow (intact cats). Pulsatile and mean CO were recorded by a Nycotron square wave flow meter (type 372, Nycotron A/S, Norway).

Epon Embedding.

Formula used for epon :

NMA	57.6g
DDSA	28.2g
Epon 812	117.2g
DMP 30	2.8ml
Total	200.0g

NMA, DDSA and Epon were mixed thoroughly for 15 min, then DMP 30 was added and mixing continued for a further 15 min.

A slightly modified epon mixture was used for embedding of freeze-dried tissue. The proportion of NMA to DDSA was decreased to soften the plastic slightly and make a suitable embedding mixture for these samples.

The mixture used contained:

NMA	55.6g
DDSA	30.2g
Epon 812	117.2g
DMP 30	2.8ml

These compounds were mixed as before.

Sørensens Phosphate Buffer (0.1M).

Solution A	$NaH_2PO_4 \cdot H_2O$	27.6 g/l distilled water
Solution B	$Na_2HPO_4 \cdot 12\ H_2O$	71.6 g/l distilled water

Solution A (ml)	Solution B (ml)	pH of Buffer
5.3	94.7	8.0
8.5	91.5	7.8
13.0	87.0	7.6
19.0	81.0	7.4
28.0	72.0	7.2
39.0	61.0	7.0
51.0	49.0	6.8
73.5	26.5	6.4

19.0 ml solution A + 81.0 ml solution B diluted to 200 ml with distilled water gives a buffer of molarity 0.1 M and pH=7.4. (Hayat, 1970).

1.7% Glutaraldehyde in Sørensens Phosphate buffer (0.08 M).

160 ml 0.1 M Sørensens phosphate buffer

14 ml 25% glutaraldehyde

26 ml distilled water

200 ml 1.7% Glutaraldehyde buffer solution.

pH=7.35

3% Glutataldehyde in Sørensens Phosphate buffer.

160 ml 0.1 M Sørensens Phosphate buffer

24 ml 25% glutaraldehyde

16 ml distilled water

100 ml 3% Glutaraldehyde buffer solution.

Tyrode

Solution A : 8.0 g NaCl

0.2 g KCl

0.05g $NaH_2PO_4 \cdot H_2O$

1.0 g glucose . H_2O

Dissolved in 300 ml dist. water.

Solution B : 0.6 g $CaCl_2$

0.3 g $MgCl_2 \cdot 6 H_2O$

Dissolved in 300 ml dist. water.

Solution C : 1.0 g $NaHCO_3$ in 100 ml dist. H_2O.

Final Tyrode solution :

9 parts solution (A+B) + 1 part solution C + 10 parts dist. water i.e.

22.5 ml solution A

22.5 ml solution B

5.0 ml solution C

50.0 ml dist. water

100.0 ml Tyrode solution

Tyrode pH = 7.47

Osmolarity = 210mOsm.

Ion concentrations in all solutions were checked by a flame photometer. pH and osmolarity were always measured.

Substances used.

Nembutal. Sodium pentobarbitone stock solution was 60 mg/ml. In anaesthesia for lung donor animals a 1:2 dilution in saline was used.

Prostaglandins E_2 and $F_{2\alpha}$. These were kindly supplied by Dr. J. Pike of the Upjohn Co., Kalamazoo, Mich., USA. Stock solutions of PG were made up in 100% ethanol and stored at -20°C. Calibrating solutions were made up daily in isotonic saline.

Metiamide. N-Methyl-N2-[(5-methylimidazol-4-yl)methyl thiol] ethyl thiourea was dissolved in 1.0N HCl and the pH returned to 6.0 by the addition of 0.1N NaOH and water. Further dilutions were made with saline. Metiamide was a generous gift from Dr. Owen, Smith Kline and French Ltd., Welwyn Garden City, Herts, England.

Bradykinin, Sandoz.

Angiotensin II ("Hypertensin") Ciba-Geigy Ltd.

Mepyramine maleate

Hyoscine hydrobromide

Phenoxybenzamine

Propranolol hydrochloride

Methisergide bimalate

Indomethacin

Glucose

Dextran T 70

"Macrodex" 6%, Pharmacia, Sweden

Heparin "novo"

Papaverine sulphate

Paraplast. Sherwood Medical Industries, St. Louis, Mass. 63103, USA.

Dammer Xylene , Searle Ltd.

Epon 812, DDSA, NMA and DMP-30 were obtained from Ladd Industries. "Epon" was stored in batches of approximately 20 ml in the deep freezer at -20°C.

DDSA, dodencenyl succinic anhydride

NMA, nadin methyl anhydride

DMP-30, 2,4,6-tri-(dimethyl aminomethyl) phenol

Alloferin (alcuronium chloride) Hoffman la Roche Ltd.

Collagen fibrils. Sigma

Ammonium oxalate

Osmium

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